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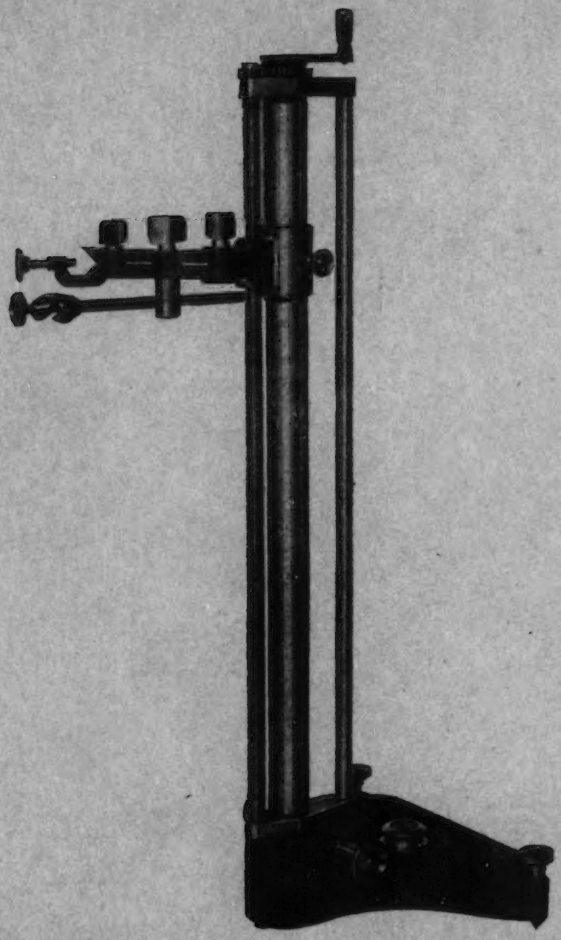

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THE VALIDITY OF THE ETHYL IODIDE METHOD FOR MEASURING THE CIRCULATION

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From the Laboratory of Applied Physiology, Sheffield Scientific School, Yale University

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Two years ago we described and applied a method for determining the circulation in man by inhalation of very dilute vapor of ethyl iodide (1). Papers reporting experience with this method have since been published also by Mobitz and Grosse (2), Cullis, Rendel and Dahl (3), Davies and Gilchrist (4), Starr and Gamble (5), Ringer (6), Rosen and White (7) H. Barcroft (8) and Moore, Hamilton and Kinsman (9). The first five of these papers report measurements of the normal circulation of the same magnitude that we obtained. On the whole they confirm the practicality of the method and the regularity of its results. It is shown also, particularly in the paper of Ringer, that in ambulant cases of heart disease, as we have found, the circulation is considerably decreased and the arterio-venous oxygen difference considerably increased. Mobitz and Grosse have carried out comparable determinations by the ethyl iodide method and by the Fick procedure which indicate that, in conformity with our conclusion, the correct practical coefficient of distribution for ethyl iodide, the factor by which the concentration in the alveolar air should be multiplied in order to give the amount taken up by the blood, has the value 2.

The last two of these papers (8, 9) contain critical discussions, but little new experimental data, bearing particularly on the accuracy of the determinations of the alveolar air by automatic sampling. A paper by one of us has meanwhile demonstrated that automatic sampling (10) affords a fair average of the so-called inspiratory and expiratory samples of the standard method of obtaining alveolar air by sudden deep expiration. We are prepared to concede to these authors, and to any future critics who may apply a strong logical imagination to problems in the field of respiratory technique, that innumerable objections are easily thought of; but they are not susceptible either of proof or disproof merely by reasoning. The possibilities of nature, at least in this field, are too numerous. It is only on

the basis of observational and experimental data collected for the particular purpose in hand that a reasonable answer can be obtained. The practical question is: Does the conception work? The first five papers above mentioned seem to answer that question in regard to the ethyl iodide method in the affirmative. Additional evidence to the same purport will be presented in this and two following papers.

Difficulty has been experienced by a number of investigators in the use of iodine pentoxide for the analysis of ethyl iodide; but nearly everyone seems, after the acquisition of some experience, to be able to obtain analytical data in which the relative amounts of ethyl iodide in various samples of air—inspired, expired and alveolar— are correctly shown. This relation is all that is necessary. A simple procedure (11) for checking the analytical reliability of the method has been published by one of us.

Starr and Gamble have developed for the analysis of ethyl iodide another method, in which the substance is decomposed by means of silver nitrate in nitric acid, and the resulting silver iodide is determined by titration with potassium sulphocyanate. We have tried this method and find it accurate. But in our hands the analysis by means of iodine pentoxide and titration with thiosulphate, as we have described it, is easier, more rapid, and sufficiently accurate. It is not worth while to press for accuracy of analysis beyond a certain point, in view of the inaccuracies, which may be as large as 5 per cent, inherent in the determination of the volume of breathing and of the pulse rate; for both these measurements have to be combined with the analytical figures, either explicitly or implicity, in practically all methods for calculation of the circulation.

Starr and Gamble have also raised a point which is of fundamental importance in regard to the coefficient of distribution. The calculation of the circulation depends upon determining two quantities and then dividing the one by the other. The first quantity necessary is the amount of ethyl iodide absorbed from the lungs in a minute. It is obtained from measurements of the inspired concentration, expired concentration, and the volume of air breathed. The second quantity necessary is the amount taken up by each liter of blood flowing through the lungs. To obtain this latter quantity it is necessary to determine the concentration of ethyl iodide in the air in the lungs. Gaseous equilibrium between the arterial blood and air in the lungs is assumed. The figure for the concentration in the alveolar air is, therefore, multiplied by a constant to afford the amount taken up by the blood. The value for this coefficient of distribution which we adopted and which, as already mentioned, Mobitz and Grosse have confirmed, is 2.

It will simplify what follows if it is kept in mind that the coefficient which is needed for practical purposes is not a figure defining the relation of the alveolar concentration to the arterial concentration, but the figure that defines the relation of the alveolar concentration to the difference be-

tween the concentrations in the arterial and venous blood. This is the amount that each liter of blood takes up each time that it passes through the lungs. Starr and Gamble show that blood which is fully equilibrated with ethyl iodide in vitro at body temperature finally takes up between 7 and 8 times as much as is then contained in an equal volume of the air to which it is exposed. They find the same relation in vivo between the alveolar air and arterial blood. They have extended, and in some important particulars they have corrected, our observations. We have confirmed their analytical data,but find quite often a value as high as 9. We have also extended their line of observation by determining the distribution of ethyl iodide between blood and air at 20°C. The figure for the distribution in vitro at this temperature rises to about 17.

Nevertheless for the reasons now to be stated, the conclusion seems to us to be unescapable that, when the figure 2 is applied to the alveolar air, it affords correctly the amount of ethyl iodide taken up by the blood in each passage through the lung, and thus leads to a correct measurement of the circulation.

Relation of alveolar concentration of ethyl iodide to the arterial concentration and to the arterio-venous difference in concentration. In our previous paper we noted that blood, in addition to the amount of ethyl iodide which it takes up in the lungs and gives off in the tissues, seems to effect a considerable additional destruction, or at least disappearance, of the substance. The coefficient of distribution of ethyl iodide between the vapor and a solution of the substance in water at body temperature is a little above 2; and for all gases blood takes up in simple solution somewhat less than does an equal volume of water. When blood is exposed to a very dilute vapor of ethyl iodide for various periods, none of them more than a few seconds, but with very full exposure during this time, the amounts taken up approximate 2. When, however, the exposure is prolonged, increasing amounts disappear. We had supposed that this must involve some slow destruction of the substance; but this supposition Starr and Gamble have demonstrated to be incorrect. From a study of this matter, which we have carried on recently and which is not yet complete, it appears that the blood holds two distinct quantities of ethyl iodide. One of these is in simple solution, and is taken up and given off virtually instantaneously when the blood is freely exposed to air. The second and additional amount is taken up more slowly and becomes fixed rather firmly in the blood, somewhat as carbon monoxide would be. It appears to be held chiefly in the corpuscles; it is probably in combination with hemoglobin, but possibly also in a dissociable compound with some of the sulphur-containing substances recently discovered. Until we have investigated this matter further we wish expressly to refrain from taking any definite stand regarding the nature of this combination, further than to point out that it seems to take no appreciable part in the

transportation of ethyl iodide from the lungs to the tissues. It is completely formed during the first 2 or 3 minutes of inhalation of ethyl iodide; its dissociation after termination of inhalation seems to require considerable time, a half-hour or longer. On the other hand the passage of ethyl iodide into and out of solution in the blood as it passes through the lungs and tissues is as rapid as that of carbon dioxide. Thus, although the venous blood returning to the right heart does contain a considerable amount of ethyl iodide, it appears to be all or nearly all combined. It is carried at a partial pressure so low as to have no considerable influence upon the amount taken up in simple solution as the blood passes through the lungs. Evidence for this conception is afforded by the fact that, as we reported previously and as we have since confirmed, no considerable concentration of ethyl iodide develops in air injected into the abdominal cavity. In accord with this fact also is an observation of Ringer: At the end of a period of inhalation, the lungs were quickly ventilated with fresh air, and then a brief rebreathing experiment was performed, so as to obtain a (Plesch) sample of venous pulmonary air. The concentration of ethyl iodide in this air was only about one-tenth of the alveolar concentration during the previous period of inhalation. Evidence to the same effect is afforded by the following experimental observations.

Concordance of circulation measurements based on application of the factor 2 to the alveolar air with measurements based on the arterio-venous difference. The experiments were carried out on large dogs. The animals were rather heavily morphinized and the carotid artery, jugular vein and trachea were exposed and opened under cocaine. A concentration of ethyl iodide vapor in air four or five times as high as that used in experiments on man was made up in a large spirometer and kept thoroughly mixed by means of an electric fan. The amounts employed were 1 cc. of liquid ethyl iodide volatilized in 100 liters of air. Arterial blood was taken from the carotid; venous blood was drawn from the right heart by means of a sound inserted through the right jugular vein. We regard this as a much more reliable technique than the insertion of a hypodermic needle into the right heart through the body wall, for it is easy for the needle to pass through the septum; in that case some of the blood may be drawn from the right heart and the rest from the left. Alveolar air was obtained by the automatic method. In our previous work 3 or 4 per cent of carbon dioxide were added to the inspired air and produced a volume of breathing which made automatic sampling more reliable. Unfortunately this precaution was omitted in the experiments here reported, and the alveolar samples were in consequence somewhat less satisfactory. The air samples analyzed for ethyl iodide were of a volume of 50 cc. The samples of blood were 10 cc. In order to extract ethyl iodide from blood very thorough aeration is necessary; the blood is blown into foam for several minutes and the corpuscles

are thus more or less disorganizing. This air current then passes through iodine pentoxide, and the iodine thus liberated is caught in KI solution. Even with this precaution analyses of blood are less exact than those of the vapor of ethyl iodide in air. The errors of analysis are magnified by the fact that the analytical data have to be multiplied by five in order to render them comparable with the analyses of air samples.

In table 1 are abbreviated the data of several experiments. In the second column are shown the amounts of ethyl iodide absorbed by the animals per minute in terms of cubic centimeters of a solution of thiosulphate used throughout each experiment, but not always in succeeding experiments. Next are given the concentrations of ethyl iodide found in the alveolar air, and then the differences between the arterial and venous

TABLE 1

Comparing the figures for the circulation calculated (1) by dividing the amount of ethyl iodide absorbed by twice the alveolar concentration, and (2) by dividing the same amount by the difference in the content in the arterial and venous blood; also some determinations by the Fick method

ANIMAL	AMOUNT OF ETHYL IODIDE ABSORBED PER MINUTE	ALVEOLAR CONCENTRATION	DIFFERENCE BETWEEN ARTERIAL AND VENOUS CONCENTRATIONS	CIRCULATION CALCULATED FROM ALVEOLAR FIGURE	CIRCULATION CALCULATED FROM ARTERIO-VENOUS DIFFERENCE	CIRCULATION CALCULATED BY FICK METHOD
A	33.0	9.2	16.0	1.83	2.05	
B	28.5	6.4	11.0	2.23	2.59	2.42
C	5.8	3.6	8.0	0.80	0.73	0.75
Same 50 minutes later	10.0	8.8	17.0	0.57	0.59	0.73
D	15.6	4.6	11.0	1.7	1.4	1.66
E	13.15	3.8	8.5	1.73	1.56	
F	9.68	3.5	7.5	1.38	1.29	
Averages		5.7	11.3	1.46	1.46	

concentrations. From these data circulation rates are calculated by our customary method of multiplying the alveolar figure by 2 and dividing it into the amount of ethyl iodide absorbed. For comparison in the next column are given the figures for the circulation calculated by dividing the arterio-venous difference into the amount of ethyl iodide absorbed. Finally for the sake of a control in some of the experiments the results of determination of the circulation by the Fick method are added.

Constancy of figures for the alveolar and expired concentrations of ethyl iodide vapor and the significance of this constancy. When any volatile substance which is not decomposed in the body, such as ethyl ether (12) or acetylene (13), is inhaled for a considerable time, the tissues of the body gradually become saturated. The venous blood returns to the lungs carrying

increasing amounts. The difference between the amounts in the arterial and venous blood decreases correspondingly. At the same time the concentrations in the alveolar and expired air rise, until at the point of equilibrium they equal the concentration in the inspired air. Such is not the case when ethyl iodide vapor is inhaled. The following experiment shows that a man may breathe a uniform concentration of ethyl iodide for forty minutes, and that throughout the whole of this time the alveolar and expired concentrations of ethyl iodide remain nearly constant, except for a slight lowering depending upon the decrease in the inspired concentration owing to absorption in the spirometer from which the air is inhaled. During so prolonged a period of quiescence the circulation, respiration and

TABLE 2

Data of an experiment showing the constancy of conditions even during prolonged inhalation of ethyl iodide

Subject: S. K. W., male, 70 kilos.

During a period of 40 minutes the subject, seated in a chair, inhaled the usual concentration of ethyl iodide,—0.2 cc. liquid ethyl iodide volatilized in 100 liters of air. At intervals samples of the inspired, expired and alveolar air were analyzed. The volume of air breathed per minute was noted and the pulse was counted.

TIME	RESPIRATION PER MINUTE	PULSE	CONCENTRATION OF ETHYL IODIDE (MEASURED IN CUBIC CENTIMETERS OF THIO)			CIRCULATION PER MINUTE
			Inspired	Expired	Alveolar	
minutes	*liters*		*cc.*	*cc.*	*cc.*	*liters*
0		82				
5	8.7	90	26.5	14.4	8.5	6.20
10	8.7		26.3	15.2	8.0	6.03
15	8.3	86	25.9	14.8	7.9	5.83
25	7.9		25.4	14.3	8.2	5.35
35	8.0		24.8	13.8	7.7	5.70
40	8.1	86	24.6	14.0	8.0	5.36

metabolism (the last not shown here) naturally diminish somewhat, and are affected by any movements due to restlessness or the discomfort of a somewhat cramped position.

We have performed such experiments as this repeatedly and on several different subjects. During rest the results are always of the character here shown. The only divergence occurs when a prolonged inhalation, or a series of inhalations in close succession, is made under conditions of vigorous muscular exercise with correspondingly augmented volume of breathing and rate of absorption of ethyl iodide. Under these conditions a point is sometimes reached at which the alveolar and expired concentrations show a distinct rise. This rise is not due to a decrease in the circulation, for the condition of the subjects—young men riding a stationary bicycle vigorously

—rules out that possibility. We infer, therefore, that after a succession of determinations involving a large absorption of ethyl iodide sufficient accumulation occurs to invalidate the experimental data. But this is true only under such extreme conditions, for one determination, or even two, lasting only five or ten minutes during vigorous physical exercise, with the allowance of fifteen minutes between inhalations, appears not to involve any appreciable error of this sort.

CONCLUSIONS

The recent papers of other observers who have used the ethyl iodide method are here briefly reviewed. It is pointed out that they afford confirmation of the practicality and reliability of the method.

The question of the proper value to be assigned the coefficient of distribution of ethyl iodide between the vapor phase and the substance in solution in the blood is discussed. It is shown that the relation of the alveolar concentration to the arterio-venous difference of concentration, that is, to the amount in simple solution, is correctly represented by the figure 2.

The constancy of conditions during inhalation of ethyl iodide, in contrast to those during saturation of the body with a physiologically nonreactive gas, such as ether or acetylene, is demonstrated. The advantage of this constancy for the use of ethyl iodide for measurement of the circulation is pointed out.

Note: Since this paper was written, additional evidence strongly supporting the validity and reliability of the ethyl iodide method has been published also by Mobitz and Hinsberg (14) and by Mobitz (15).

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COMPARATIVE MEASUREMENTS OF THE CIRCULATION IN MAN WITH CARBON DIOXIDE AND WITH ETHYL IODIDE

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The relation of the circulation to the respiratory metabolism, that is, the relation of the volume of blood pumped by the heart per minute to the volume of oxygen consumed by the body and the amount of carbon dioxide produced, is one of the most fundamental of physiological adjustments. It would be of great practical importance to know, and to be able to determine on each individual, how this relation varies during rest and exercise, in the trained athlete, in the man of sedentary habit, and in the cardiac patient. But before that problem is undertaken, we must determine the relation under the simplest conditions, those of normal men at rest.

In most textbooks even now the difference in the content of oxygen in arterial and venous blood is stated as 7 or 8 volumes per cent. When we correlate this statement with an oxygen consumption of 240 cc. per minute, such as a normal man has in the basal condition, the circulation works out to $\frac{240}{0.08} = 3000$ cc. per minute. Even in important monographs published within the last year or two by leading investigators in the field of the hemato-respiratory and circulatory functions, the arterio-venous oxygen difference is referred to as probably about 6 volumes per cent. Six volumes per cent would correspond to an estimate of the circulation of $\frac{240}{0.06} = 4000$ cc. per minute. Against this view recent papers from this laboratory have presented data, and have reviewed the observations of others, in order to show that the arterio-venous oxygen difference in a healthy man during rest is probably about 4 volumes per cent and his circulation, if we assume 240 cc. per minute oxygen consumption, is therefore $\frac{240}{0.04} = 6000$ cc.

This is a matter of great general importance. Questions of the validity or defects of various methods of determining the circulation are in comparison of quite minor importance. If the normal circulation is so large

that the blood on the average gives up only 4 volumes per cent of oxygen in passing from arteries to veins, then the tissues live in a pressure of oxygen which is much higher than it would be if the blood flow were only two-thirds as large. The reserve upon which the body can draw at need, both for exertion and in disease, is correspondingly greater.

The sum total of all available evidence indicates, we believe (1), that an arterio-venous oxygen difference not appreciably above 4 during bodily rest is an essential of every vigorous healthy man. If the figure is 6, the limitation on vigor is equivalent to that from which an ambulant cardiac patient suffers. As an approximation, we infer that, if the difference is 8, the condition of the patient would confine him to his bed or at best to a wheel chair. He would be capable of very limited exertion indeed. He would be in the state of the animals under experimental conditions, anesthetized, operated, and therefore more or less shocked, in which arterio-venous differences of 8 volumes per cent, or larger, are found. It is in fact observations under these conditions that have afforded the basis for the erroneous figures heretofore generally accepted.

The alleged discordance of results. There seems to be a rather widely disseminated opinion that the ethyl iodide method yields results indicating larger circulations than other methods based on the normal respiratory gases; and in particular larger than the procedure employed by Bock and his collaborators (2). We are not concerned to discuss the merits or demerits, the accuracy or inaccuracy of that procedure; for skill and care might obviate defects, if there are any. But it is of fundamental importance to know whether measurements of the circulation on any particular set of men by means of carbon dioxide and by means of ethyl iodide yield systematically different values.

Before discussing the data which we have to present on that question there are three propositions to which we wish briefly to direct attention.

The first proposition is a matter of the history of the development of this subject. It is, we think, a complete misnomer to speak of the determination of the circulation in man based on the respiratory gases as one made by the "Fick method," or "Fick principle." Fick never imagined anything of the sort. The utmost that he did was to point out, for he made no experiments, that if we have given figures from analyses of the gases of arterial blood and average venous blood together with the respiratory exchange, the circulation may be calculated. Such a determination should really be called the Zuntz method for it was Zuntz who put it into practice; but it is scarcely worth while now to reverse the custom of referring such measurements on animals to Fick.

A more important point is that determinations of the circulation in man based on the respiratory gases should not be denominated either as the Fick or the Zuntz method; for it is not possible to obtain mixed venous

blood from the right heart of a man. Only on animals can a needle or catheter be inserted. The determinations on man involve a large additional conception which was first put forward in the classic paper of Christiansen, Douglas and Haldane (3). They demonstrated that, if air is obtained from the lungs in gaseous equilibrium with arterial blood, and also air in equilibrium with the venous blood, the partial pressures of oxygen and carbon dioxide in these airs can be applied to the dissociation curves of the blood. From the points thus fixed in those curves they inferred the gaseous contents of the blood in volumes per cent. This procedure would not have been possible prior to the work of Christiansen, Douglas and Haldane, for the reason that these investigators were the first to plot the CO_2 dissociation curve and to demonstrate the influence of the pressure of oxygen upon its level. If, therefore, any investigator's name should be attached to determination of the circulation in man by means of the normal respiratory gases it should be called the Haldane method.

The second proposition may seem to compromise to some extent, but only on a matter of detail, the credit thus assigned. Part of the idea upon which Christiansen, Douglas and Haldane worked was to produce in the lungs, and then to obtain from them, a gas mixture in equilibrium with the venous blood coming from the right heart. This seemed to them to be necessary, in order that the interaction of oxygen and carbon dioxide should be allowed for, and false estimations of pressure avoided. This line of thought has been followed by most of the investigators who have since tried to apply the Haldane method. But experience in this laboratory leads us to believe that its demands cannot be fulfilled with certainty. In order to get down to the oxygen pressure of the venous blood, a gas mixture of extremely low content of oxygen must be inhaled. This technical difficulty can indeed be overcome, but there is no adequate criterion for knowing whether, or with what degree of accuracy, the end sought has been accomplished. Some investigators have made use of semi-expiration or a first alveolar sample, followed by full expiration some seconds later; but even the requirement of concordance between these samples is not sufficient evidence that the venous pressures have been exactly balanced, for the first sample comes chiefly from the dead space. We shall not, however, argue this point further, but will leave the matter with this expression of opinion: It is not possible to base accurate determinations of the circulation upon the oxygen pressure and content of the venous blood in any form of the Haldane method.

The third proposition is that the Haldane method can be made effective and fairly accurate, but only on trained subjects and only during rest and moderate exertion, by eliminating the oxygen effect and basing the calculation of the circulation wholly upon the pressures of carbon dioxide (4).

As regards the arterial blood, there can be no objection to this. It is nearly saturated with oxygen and the pressure of carbon dioxide is obtainable from the alveolar air or, as it would be more precise to call it, the "arterial pulmonary air." As regards the venous blood, however, with its reduced content and pressure of oxygen, simply to neglect the oxygen pressure would introduce an error of unknown magnitude into calculations based on the pressure of carbon dioxide in venous pulmonary air obtained by the Plesch technique of rebreathing into a bag. Accordingly in this laboratory we have adopted the procedure of filling the Plesch bag with oxygen plus enough carbon dioxide to balance the pressure of this gas in the venous blood *after the blood has been fully oxygenated.* When such a mixture is rebreathed for twenty seconds, the venous blood is fully oxygenated, but it neither takes up nor gives off carbon dioxide. The pressure of carbon dioxide in the blood in the lungs, and in the gas mixture in equilibrium with it, is then decidedly higher than the pressure of carbon dioxide in the partially deoxygenated blood in the right heart. The advantage of relying upon this pressure of carbon dioxide—not the pressure in true "pulmonary venous air" but that in "virtual venous air"—lies in the fact that the value so obtained may be applied directly to the CO_2 dissociation curve of fully oxygenated blood. Another advantage is that an excellent criterion of the reliability of the virtual venous value is obtained by a series of rebreathings of various durations.

We have thus two points, each defined in units of pressure, which can be applied on the carbon dioxide dissociation curve of *fully oxygenated* blood, so as to afford the corresponding quantities of carbon dioxide in volumes per cent in the arterial and venous bloods respectively. Furthermore in practice it is not necessary to deal with these two points, but merely with the difference between their values. The level of the dissociation curve varies with the amount of the blood alkali. But its slope, as has been pointed out previously (5), is nearly the same in all normal bloods; for it is determined by the amount of hemoglobin. Thus, for each rise of 1 per cent of an atmosphere in the pressure of carbon dioxide, fully oxygenated normal blood takes up, with little individual variation, 3.42 volumes per cent of carbon dioxide. In observations upon normal men, it is not only much easier to use this factor for converting differences of pressure into volumes per cent than it is to equilibrate and analyze blood samples; it is also on the whole more accurate, since the slope of the curve is known from many analyses.

It is in the form thus indicated that we have employed the Haldane method for measuring the circulation.

Concordance of results. For comparison we have measured the circulation on the same subjects by means of ethyl iodide (6) and by means of carbon dioxide. The subjects were not always under basal conditions;

and they merely sat still for 5 or 10 minutes before each test. As the two methods of measurement could not be carried out absolutely simultaneously the output of carbon dioxide was determined in connection with the

TABLE 1

Showing comparable measurements of the circulation by means of CO_2 and by means of ethyl iodide

		R. J. B.		H. W. H.		Y. H.	
		CO_2	E. I.	CO_2	E. I.	CO_2	E. I
CO_2 production (cubic centimeters per minute at S. T. P.)	(a)	197.0	197.0	241.0	272.0	249.0	238.0
	(b)	220.0	215.0	287.0	289.0	271.0	256.0
	(c)	228.5	228.5	288.5	296.0	286.0	275.0
	(d)	234.0	231.0				
Arterio-venous CO_2 difference (volumes per cent)	(a)	3.59	3.31	3.42	3.58	3.42	3.15
	(b)	3.76	3.85	3.66	4.39	3.86	3.42
	(c)	3.84	4.72	3.90	4.07	3.80	3.48
	(d)	3.90	5.13				
Circulation (liters per minute)	(a)	5.48	5.95	7.04	7.62	7.27	7.56
	(b)	5.84	5.59	7.84	6.59	7.01	7.50
	(c)	5.95	4.85	7.40	7.27	7.52	7.91
	(d)	6.00	4.50				
Pulse	(a)	72.0	72.0	87.0	78.0	63.0	63.0
	(b)	75.0	72.0	82.0	82 0	62.0	66.0
	(c)	87.0	75.0	84.0	84.0	62.0	68.0
	(d)	84.0	72.0				
Stroke volume (cc.)	(a)	76.0	82.7	81.0	97.9	115.5	120.0
	(b)	78.0	77.6	95.7	80.3	113.3	114.0
	(c)	68.4	64.7	88.2	86.5	121.1	116.3
	(d)	71.4	62.5				
Stroke index (cubic centimeters per kilo per beat)	(a)	1.23	1.33	0.90	1.09	1.41	1.46
	(b)	1.26	1.25	1.06	0.89	1.38	1.39
	(c)	1.10	1.04	0.98	0.96	1.48	1.42
	(d)	1.15	1.01				
Dead space (per cent)	(a)	35.4	31.8	34.3	34.5	22.1	37.9
	(b)	28.3	33.3	25.4	38.0	31.1	34.5
	(c)	35.1	34.5	25.1	33.9	24.9	26.7
	(d)	27.6	40.0				

measurements by means of ethyl iodide; and the arterio-venous carbon dioxide difference was then calculated for these experiments by dividing the carbon dioxide output by the minute volume of the circulation. The

essential data and results in as nearly as possible comparable experiments by the two methods are shown in the accompanying table.

The two methods are here seen to afford results in reasonable agreement with each other: a fact which affords strong support to the validity of the value 2 for the factor by which the alveolar concentration of ethyl iodide is multiplied in the ethyl iodide method for measuring the circulation (7). As all of the observations were made a couple of hours after a light breakfast it is fair to assume a respiratory quotient of a value of about 0.8. On this basis the average arterio-venous oxygen difference for all the observations here reported would be a quarter larger than the figures for the

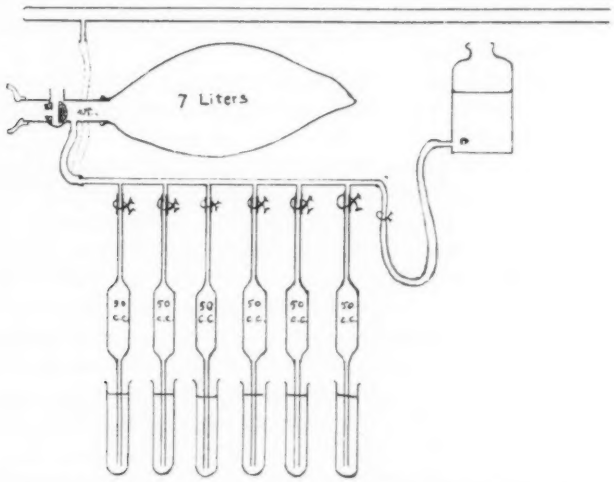

Fig. 1. Apparatus used to obtain "arterial pulmonary air" and "virtual venous air" for determination of the arterio-venous CO_2 pressure.

carbon dioxide difference shown in the table and would average to a value a little above 4 volumes per cent.

Details of method of determining the circulation by means of carbon dioxide. After sitting at rest for a period of at least 5 minutes, and until the pulse became regular, the subject's expired air was collected for five to ten minutes in a Douglas bag, metered and analyzed, and the CO_2 production determined. Without shifting his position, the subject then commenced breathing through the mouthpiece of the apparatus shown in figure 1. The bag (of capacity 7 liters) had previously been partially filled with about 6 liters of a mixture of oxygen and approximately 5 or 6 per cent CO_2. After the deepest possible expiration, the tap was turned and the subject inhaled deeply from the bag and breathed back and forth into it for 30

seconds, finishing with a deep expiration into the bag. After an interval of at least 1 minute, when the breathing had become quite regular again, the rebreathing process was repeated with the contents of the bag for another 30 seconds. Then again intervals of rest were followed by periods of rebreathing of 25 seconds, 20 seconds and 15 seconds. At the end of each rebreathing period, except the first, a sample of the contents of the bag was drawn into one of the 50 cc. sampling tubes by opening the appropriate spring clip. Before the experiment the sampling tubes had been completely filled with water, and before the collection of each sample the whole of the system of tubes was filled up to the level of the bag by raising the water reservoir. These samples were analyzed for CO_2, and represent specimens of virtual venous air. Even the last one usually contained more than 25 per cent of oxygen.

Without shifting his position, the subject finally gave samples of his alveolar air by means of a Haldane-Priestley tube, the samples being collected as before.

The method of calculation was to subtract the average CO_2 figure for the "virtual venous air" from the average arterial CO_2 figure. This gives the arterio-venous CO_2 difference in percentage of an atmosphere. This figure was then multiplied by the pressure-volume factor, and gave the arterio-venous difference in volumes per cent of CO_2. The latter figure was divided into the CO_2 production; and the quotient was the volume of blood passing through the lungs per minute, that is, the circulation. The stroke volume is the circulation divided by the pulse rate. The stroke index is the stroke volume divided by the body weight in kilos.

The dead space was calculated in every case as a check and criterion of the accuracy of the observations.

The following protocol illustrates these calculations.

Subject, weight 62 kgm.

Haldane-Priestley samples of arterial alveolar air contained 5.46 per cent (inspiratory), and 5.56 per cent (expiratory) CO_2.

Average CO_2 content = 5.51 per cent

Samples taken from bag (equivalent to virtual venous air) contained

(1) rebreathing 30 seconds; (discarded)
(2) rebreathing 30 seconds; 6.52 per cent CO_2
(3) rebreathing 25 seconds; 6.74 per cent
(4) rebreathing 20 seconds; 6.67 per cent
(5) rebreathing 15 seconds; 6.66 per cent

Average CO_2 content = 6.65 per cent

Arterio-venous CO_2 difference = 6.65 − 5.51 = 1.14 per cent

corresponding to an a-v CO_2 difference of (3.42 × 1.14) = 3.90 volumes per cent

CO_2 production at rest, per min. 234 cc. at S. T. P.

$$\text{Circulation rate} = \frac{0.234}{3.90} \times 100 = 6 \text{ liters per min.}$$

$$\text{Stroke Volume (pulse} = 84 \text{ per min.)} = \frac{6000}{84} = 71.4 \text{ cc.}$$

$$\text{Stroke Index (body weight} = 62 \text{ kilos)} = \frac{71.4}{62} = 1.15 \text{ cc. per kgm.}$$

Dead space.

$$\frac{\text{Alv. } CO_2 - \text{Exp. } CO_2}{\text{Alv. } CO_2} =$$

$$\frac{5.51 - 3.99}{5.51} = \frac{1.52}{5.51} = 0.276$$

$$0.276 \times 100 = 27.6 \text{ per cent}$$

CONCLUSIONS

It is here shown that determinations of the circulation by means of ethyl iodide and by means of carbon dioxide afford values of the same magnitude. Both methods indicate that the circulation in healthy men at rest is so large that the arterio-venous CO_2 difference is on the average only about 3.5 volumes per cent and the oxygen difference therefore (on an R. Q. of 0.8) only a little above 4 volumes per cent.

It is pointed out also that determinations of the circulation by means of the respiratory gases should properly be called not the Fick, but the Haldane method. Reasons are here stated why we do not believe that measurements of the circulation based on determinations of the oxygen pressure in the venous blood coming to the lungs are practically feasible. On the other hand the use of the figures for the arterial and virtual venous carbon dioxide pressures—the latter being the pressure of carbon dioxide in the venous blood after saturation of oxygen—affords the simplest form of the Haldane method.

Note: A recent paper by Dautrebande (8) confirms the reliability and accuracy of the method here used to measure the circulation by means of CO_2.

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THE EFFICIENCY OF THE HEART, AND THE SIGNIFICANCE OF RAPID AND SLOW PULSE RATES

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The efficiency of the heart is nothing else than the volume of blood that it can pump in relation to the oxygen requirement in the body. This applies alike to the athlete, to the man of sedentary habit, and to the cardiac patient. The index of efficiency is therefore the arteriovenous oxygen difference during rest and various degrees of muscular exertion. An exertion which would be moderate and would involve no appreciable oxygen debt for the man with a more efficient heart, involves (as Meakins (1) also has pointed out) the incurring of such a debt in proportion as the heart is less efficient. An oxygen debt, exceeding that which the individual can easily carry and make up, generally induces both excessive breathing and an excessively rapid pulse.

The general relations of the mammalian circulation to respiration, metabolism, energetics and body weight have been formulated by one of us in lectures (2) published two years ago. It was then pointed out that the basal circulations of vigorous mammals of all sizes are proportional to their resting oxygen consumption and proportional therefore also to their metabolism, heat production and skin surfaces. The volume of blood in the body and the weight of the heart in animals of corresponding vigor, but of different sizes, are not proportional to surface, but to body weight. Thus, to express the matter more precisely, in all vigorous mammals of all sizes (dogs, men and horses) about 100 cc. of blood flow through the lungs, and are pumped on by the heart to the tissues, for each 4 cc. of oxygen that the animal consumes during rest. The stroke index in all vigorous mammals is about 1.5 to 1.8 cc. per kilo body weight per beat. Thus, as the volume of blood in the body is proportional to the body weight, and the volume of the circulation is proportional to the skin surface, while the stroke index in all vigorous species of mammals is nearly the same, the pulse rate during bodily rest in animals of various sizes must be an expression of the ratio of surface to weight; it must be proportioned to the intensity of the resting metabolism. This inference checks satisfactorily with observation; for the pulse rates of small animals are rapid and those of large animals slow in fairly close relation to the surface-weight ratio (15).

This is the fundamental correlation of the mammalian circulation with other functions. The new fact, which now permits the correlation to be seen, is that the arterio-venous oxygen difference of a resting mammal of any vigorous species is only about four volumes per cent (3). The pressure of oxygen in the tissues and in the blood flowing from them is therefore much higher, and the third reserve of the circulation, the amount of oxygen in the venous blood, is much larger than would be the case if the circulation were smaller than it is in proportion to the oxygen consumption.

Variations in the circulation in rest, exercise and excitement. It is a much more difficult problem, and practically even more important, to determine how the circulation of an individual varies during the varying activities of life, such as muscular work, fatigue, and pleasurable excitement or anxiety. It is probable that, throughout a moderate range of muscular activity, the circulation varies more or less closely in proportion to the energy expenditure and thus to the oxygen consumption of the body. This proportionality requires and demonstrates that there is some process through which metabolism regulates the volume of the venous return, the flow in liters per minute to the right heart.

The regulation of the volume of the venous return is probably effected in some manner depending upon, or at least related to, the amount of carbon dioxide produced in the tissues at the time; but the mode of this regulation is a topic which we shall not attempt to deal with here. Nor shall we discuss the process, or processes, by which the heart rate is varied. We wish merely to recognize that both the venous return and the heart rate do vary. It is the relation of the variations of these functions to each other and their balance against the oxygen demands of the body that will be dealt with here; that is the correlation between them.

The relation of the rate of the heart to the minute volume of the circulation has not yet been clearly formulated. Obviously the heart cannot pump into the arterial system a larger volume than the venous stream brings to it. If, on the other hand, the pumping activity of the heart falls behind the volume of the venous return, congestion must immediately result: a congestion by which the jugular veins and even the veins of the forehead would be distended to an extent which we never see in a normal man, except under extreme physical exertion. This condition is often seen in heart disease; for there is then a failure of coördination of heart action and venous return. Indeed mere observation of the jugular veins of a healthy man is sufficient to demonstrate that during rest and moderate exertion there is a fairly close coördination of the two functions, but that under severe exertion the venous return may run ahead of the heart action. The neck veins are then noticeably distended (16, 17).

Perfect coördination is certainly not always maintained even in healthy

men. Although the metabolism and pulse rate may vary proportionally under some conditions, under others they go far out of such coördination. It is well known, for instance, that mental excitement, either pleasure or anxiety, in comparison to muscular work, exerts little influence upon metabolism. Even at very exciting athletic contests (e.g., football games) the spectators, who are excited but are sitting or standing still, often get very cold. So also basal metabolism determinations are frequently made on patients who are distinctly anxious, but if they are not thyroid cases a normal resting oxygen consumption is found in spite of their excitement.

On the other hand, the pulse rate is extremely susceptible to emotional influences. Every new and interesting idea, and much more every anxiety or fear, quickens the heart beat. Such emotional animals as dogs are so responsive to attention or handling by their master, and their pulse rates are so much affected that it is rather difficult to determine what their resting pulse rate really is (13).

Thus under mental excitement, when the oxygen consumption does not increase, but the pulse rate does, the functions of the venous return and the activity of the heart must go out of adjustment; the stroke volume must then be much smaller than it is at the same rapidity of pulse rate during muscular exercise. This is simple arithmetic; for the volume of blood pumped on by the heart per minute is necessarily the product of the number of beats multiplied by the volume that the single beat discharges. If the circulation is of nearly the same volume per minute during mental excitement not involving muscular exertion that it is during rest, as we have good reason to believe, the more rapid pulse necessitates smaller beats.

According to this conception of the circulation the vasomotor mechanism (with the associated adrenals), and the mechanism regulating the venous return are distinct. One of the strongest reasons for this distinction is to be found in the difference in their behavior under the influence of mental excitement. Under excitement arterial pressure rises. In this laboratory a rise of 50 to 60 mm. has been noted in students who had listened to a difficult lecture, and an even greater rise in the lecturer. Carl Tigerstedt (4) records a similar, but slightly less, rise of pressure in students taking written and oral examinations in Finland. The rise is wholly due to constriction of the peripheral arteries and arterioles by stimuli coming from the brain through vasomotor nerves and by discharge from the adrenals. Although the heart beats faster also, it does not pump into the arteries any considerably greater volume of blood per minute than it would during rest without excitement.

In striking contrast to these conditions, but testifying no less directly to the independent existence of the mechanism of the venous return from that of the vasomotor and adrenal mechanism are the effects of muscular work. During muscular work the venous stream is greatly accelerated, for otherwise the circulation could not be increased; but arterial pressure in a man in good physical condition is elevated to only a moderate degree and the arterioles of the skin and muscles are noticeably dilated.

A uniform stroke volume as the resultant of perfect coördination between heart rate and venous return. The circulation, that is, the volume of blood pumped into the arterial system per minute, is the resultant of two factors: the venous return and the heart action. The relation of these factors might be as follows: 1, the venous supply to the right heart might be at all times so ample that the heart could always take all it would. In this case the variations in the volume of the circulation would be determined wholly by the heart. Or 2, the venous return might be the variable factor, and the function of the heart might be merely to pump onward the volume supplied to it, no more and no less. Then, just as in the first case it was the heart, so in the second case the control of the venous reservoirs of the body would afford the solution of this essentially Harveyan problem. But it would be unphysiological to expect either of these extremes to prove true to the exclusion of the other. Everywhere in the normal living body we find complementary functions working in coördination. Under conditions of strain or abnormality we find that the balance is pushed in one direction or another, as one function exceeds or falls a little behind. But always two or more functions are involved in the resultant, alike when the resultant is a certain volume of flow, as in this case; or a certain arterial pressure, as between the heart and the vasomotor mechanism; or the maintenance of body temperature by heat production and dissipation; or whatever the condition to be maintained.

This conception of the coördination of the heart rate and the volume of the venous return affords an explanation for a uniform stroke volume. If a man has twice as many heart beats when doing a certain amount of work as he has during rest, and if the volume of the venous return is twice as large under the one condition as under the other, then the stroke volume will necessarily be the same during work and rest. In accord with this conception it was found by Henderson (5) in some experiments reported twenty years ago, and much disputed by others ever since, that in dogs the ventricles of the heart may discharge nearly the same volume at each beat at slow, rapid and all intermediate pulse rates: a uniform ratio of circulation to pulse. He suggested that this might be the normal relation of the activity of the heart and the venous return. But he did not regard that relation as one inherently necessary; for he devoted only one paper to it, but published in the succeeding years several papers (6) in which wide divergencies from a uniform stroke volume were described and the factors conditioning them were analyzed. In a more recent review (7) the idea of a uniform stroke volume was again emphasized. All that need be recalled now is that whenever as the result of hemorrhage, or acapnia, or cold, or other abnormal conditions due to anesthesia and operation, the adjustment of pulse rate and venous return was disturbed, the stroke volume became abnormally small. On the other hand when the animals were kept as nearly normal as possible, especially in respect to the carbon dioxide con-

tent of the body it was found that the pulse could be automatically adjusted to a wide variety of rates; but the stroke volume was uniform.

Similarly Douglas and Haldane (8) found that in some men the stroke volume is the same during work and rest, but that in other men it was increased during work.

Methods of observation on men. By means of the ethyl iodide method (9) we have measured the circulation during rest and exercise on 50 young men, nearly all students in this university, most of them 22 or 23 years of age, the youngest 18 and the oldest 25. About one-third were engaged in one or another of the more strenuous forms of competitive athletics, such as rowing. About as many more were accustomed to take exercise merely for pleasure, usually either tennis or golf. The remainder were all in good health, although not leading physically active lives. They came to the laboratory at any convenient time during school hours regardless of whether it was before or after a meal. Many of the observations were made under post-nutritive conditions, for we find that for about an hour before the midday meal this is the condition of most college students. Others were tested soon after the midday meal; and others in intermediate conditions. The respiratory quotients indicate their conditions in this respect.

The subjects always sat for ten minutes before the test and for ten minutes while the circulation and respiratory metabolism were determined. The figures in the tables giving the oxygen consumption indicate that in some cases the men were in a state of complete rest. In other cases the condition was equivalent to that of a man sitting quietly working at a desk.

The subject next mounted a bicycle ergometer and rode at a fixed rate (120 turns of the pedals per minute) and against a resistance consisting of a Pronay brake on a fly wheel so adjusted that the amount of work done was 960 kilogram meters per minute. When the pulse and respiration were steady the circulation and respiration were measured.

All of the men did exactly the same amount of work; yet the amount of oxygen consumed by them differed considerably. Most of them consumed 2.5 to 3 liters per minute; the lowest was 1.62, and the highest 3.48. That the work was not excessive is shown by the fact that the respiratory quotients during the work were not as a rule appreciably higher than during the period of rest previously. We can see no reason in these data to alter our opinion based on a study (10) of the Yale Crew three years ago that the fuel of muscular work in man consists of fat as well as carbohydrate and in nearly the same proportion as during rest. American investigators seem generally to agree in this finding as against that of A. V. Hill (11), who concludes that sugar is the sole source of the energy of muscular work.

The arterio-venous oxygen difference (or, for short, the A-V difference) expresses the amount of oxygen in cubic centimeters that each hundred cubic centimeters of blood lose in passing through the tissues. It expresses

therefore, as previously explained, the relation of the volume of the circulation to the man's total oxygen consumption at that time; it shows also the amount in reserve, the additional oxygen that he might draw from his blood for a greater exertion. In most of these young men the A-V difference during rest was found to lie between 3 and 5 volumes per cent, the lowest was 2.3 and the highest 5.1.

During the exercise which, as above stated, was 960 kilogram meters of work per minute for everyone alike, the A-V difference varied according as the work was, for the individual, quite heavy or more or less easily carried. The best showing made by anyone was an A-V difference of only 9.1 volumes per cent; the worst showing was 17 volumes per cent, a figure which is probably the maximum of which the man is capable except for a very brief period. In this man the circulation had reached its maximum at some much lower expenditure of energy; and all the additional expenditure was effected by an extreme depletion of the oxygen from the blood; for the venous blood in this case was about 90 per cent unsaturated.

It may seem that the use of the same amount of work for all of the men would not really put the athletes and those of more sedentary habits on a fair basis of comparison; for the latter class the exertion was much more of a strain than for the athletes. But we were aiming to get the circulation, not the energy expenditure, up to a maximum in all cases. From our general experience we believe that the pulse rates during work were about as high as they could go in most of the men concordantly with maximum circulatory efficiency. Harder work would not have increased it, but would have been effected by drawing on the third reserve of the circulation, that is, by a further depletion of the oxygen content of the blood in its passage through the tissues, and a corresponding increase of the A-V difference. Thus the relation of the circulation during work to the circulation during rest, as shown in the tables, expresses the range of adjustment of which the hearts of these men are capable.

Comparison of conditions inducing a uniform stroke with those inducing an increased stroke during exertion. The men whom we examined fall, as stated above, into three chief classes: those taking no regular exercise, those who take exercise which is merely vigorous, and those who engage in athletics involving violent exertion. By vigorous exercise we mean a degree of exertion that can be maintained for one or more hours steadily, as in golf or fast walking or the ordinary rate of work of a laborer (12), involving a consumption up to 1000 or 1500 cc. of oxygen per minute. By violent exercise we mean such exertion as the most strenuous forms of athletics, rowing, running, football, in which for a few minutes at a time every cubic centimeter of oxygen that the heart can supply to the muscles is demanded.

In the first class, the non-athletes, the stroke of the heart is of approxi-

TABLE 1

Data on the circulation in 10 non-athletes during sitting rest and while doing 960 kilogram meters of work per minute on a bicycle ergometer

Instead of age, weight and height, the corresponding basal oxygen consumptions per minute, according to du Bois' chart, are given in column 1. Then the oxygen consumption observed in liters per minute at 0° and 760 mm. Pulse rate. Respiration in liters per minute. Respiratory quotient after the steady state is attained. Circulation in liters per minute determined by ethyl iodide method. Arteriovenous oxygen difference in volumes per cent. Stroke volumes in liters. Stroke index in cubic centimeters per kilo body weight per beat. In each case the upper line of figures is for rest, and the lower for exercise.

BASAL OXYGEN	OXYGEN CONSUMPTION	PULSE	RESPIRATION	R. Q.	CIRCULATION	A–V DIFFERENCE	STROKE VOLUME	STROKE INDEX
0.259	0.335	74	10.00	0.80	6.7	5.0	0.091	1.28
	1.80	150	50.75	0.79	14.65	12.3	0.098	1.40
0.263	0.342	76	8.59	0.80	8.75	3.9	0.115	1.57
	2.43	166	49.50	0.87	17.89	13.6	0.108	1.48
0.237	0.241	78	6.80	0.86	8.73	2.8	0.106	1.34
	3.16	160	44.00	0.71	17.9	17.6	0.112	1.41
0.267	0.310	81	9.10	0.84	8.2	3.8	0.101	1.33
	3.000	158	49.00	0.76	18.2	16.5	0.115	1.51
0.260	0.392	88	9.20	0.78	10.7	3.68	0.123	1.76
	2.21	156	47.10	0.76	17.1	13.00	0.110	1.58
0.258	0.316	83	9.40	0.85	8.2	3.85	0.099	1.32
	2.86	162	50.20	0.80	17.4	16.5	0.107	1.43
0.262	0.268	79	7.20	0.74	9.4	2.85	0.119	1.51
	2.93	164	51.60	0.72	19.7	14.85	0.120	1.52
0.264	0.347	84	10.20	0.83	9.3	3.73	0.111	1.50
	3.16	170	61.00	0.80	18.7	16.9	0.112	1.52
0.259	0.377	87	8.00	0.85	9.3	4.0	0.107	1.44
	3.10	162	54.60	0.81	20.2	15.3	0.123	1.66
0.270	0.474	80	11.70	0.74	9.3	5.1	0.116	1.45
	2.96	182	61.20	0.77	18.4	16.1	0.101	1.26
Average increase, per cent of resting value.....	680	103	487	−3	104	275	2	2

mately (that is, within about 10 per cent) the same volume during rest and exercise. The data of 10 typical cases are shown in table 1.

The second class needs no table of illustrative data. It is sufficiently described as in all respects intermediate, between the non-athletes and athletes.

TABLE 2

Data on the circulation in 10 athletes. Arranged as in table 1

BASAL OXYGEN	OXYGEN CONSUMPTION	PULSE	RESPIRATION	R. Q.	CIRCULATION	A–V DIFFERENCE	STROKE VOLUME	STROKE INDEX
0.298	0.400	74	8.17	0.70	9.35	4.3	0.127	1.41
	2.300	132	41.25	0.71	24.50	9.4	0.186	2.06
0.295	0.423	45	10.00	0.89	9.1	4.65	0.202	2.34
	2.48	126	49.50	0.80	24.55	10.00	0.195	2.26
0.276	0.458	70	7.00	0.76	9.9	4.6	0.140	1.75
	3.00	145	66.00	1.00	32.0	9.4	0.221	2.75
0.284	0.466	62	11.0	0.84	10.1	4.4	0.163	1.92
	2.600	136	51.2	0.85	30.4	8.6	0.224	2.63
0.287	0.452	66	9.2	0.84	10.4	4.4	0.158	1.92
	3.061	130	44.7	0.80	27.4	11.2	0.210	2.56
0.272	0.430	70	8.7	0.85	9.8	4.4	0.140	1.75
	2.93	140	50.0	0.82	26.1	11.5	0.186	2.33
0.291	0.482	68	10.2	0.82	11.0	4.2	0.162	1.89
	3.01	136	46.4	0.78	26.8	11.2	0.197	2.30
0.298	0.410	60	9.8	0.85	8.8	4.6	0.147	1.65
	2.60	132	48.2	0.83	24.6	10.6	0.187	2.10
0.284	0.422	68	8.0	0.84	8.7	4.8	0.128	1.55
	2.86	142	67.0	0.92	28.1	10.2	0.198	2.40
0.258	0.401	78	9.7	0.84	7.9	5.1	0.101	1.51
	2.41	152	56.0	0.84	21.2	11.4	0.143	2.10
Average increase, per cent of resting value.....	530	104	530	6	224	128	33	33

In the third class, the athletes, the stroke volume generally increases considerably, in some cases 40 or 50 per cent or more, with exertion. The data of 10 typical cases are shown in table 2.

These differences of stroke are accompanied by differences of pulse rate which are equally significant. Athletes frequently have very slow pulses; their rates are 10 or 20 or even 30 beats slower than in men of sedentary habit. Among the men taking little exercise the slowest pulse observed was 74 and the fastest 88. Among the athletes the slowest pulse under the same conditions of sitting rest was 45 and the fastest 78.

The two groups, non-athletes and athletes, as shown in tables 1 and 2, are at first sight alike in that in both the pulse was about twice as rapid during exertion as during rest. But this similarity is superficial. When a man out of training exerts himself so that the pulse rate is doubled, his circulation is usually merely doubled also. When the athlete's pulse rate is doubled, his circulation often undergoes an increase to three or more times the resting value. Thus fundamentally the doubling of the pulse increases the difference between the two classes during exertion. Twice a resting pulse of 60 is 120 beats per minute; twice one of 80 is 160; twice one of 90 is 180. A pulse of 120 during work, such as an athlete may have, is associated with no sense of strain; but one of 180, as in a man out of training, is near the extreme upper limit of efficient work for a heart of the size of that of man. At rates above 180 the efficiency decreases progressively, for the diastoles then become too brief to allow the ventricles to relax and refill. It is the relatively slow full pulse of the athlete which enables his heart to supply the blood stream and transportation of oxygen for a four mile boat race or a Marathon run. In such contests the athletes do not become "winded," as untrained men would; they carry on to the point of exhaustion. Their condition then is not due to excessive accumulation of lactic acid, as it is in short maximal exertions. It is probably the deficiency of available sugar that puts a limit on their activity.

Second wind and fatigue. The phenomenon of "second wind" is one that has always excited interest. It includes several factors, respiration, sweating and circulation. Each of these factors after an initial period of somewhat excessive activity comes into balance with the continuing demand upon it at a more moderate level. As regards the pulse and stroke volume of the heart this initial condition of strain and the reëstablishment of balance in second wind are illustrated by the first case shown in table 3. Here the pulse had risen initially to 144; but dropped to 126 as the demand and supply for oxygen became balanced. As the man tired his pulse rose again, reaching 165; and this rise was accompanied by a decreased stroke volume. He was approaching exhaustion.

The second and third cases in table 3 show likewise excessive pulse rates during exercise and the consequent decrease of stroke volume.

An even more striking example of the decreased output of the heart in some untrained individuals at rapid pulse rates associated with fatigue is shown in the last case in table 3. In this man during exertion the

pulse increased to 246 beats per minute. In this extreme condition the stroke volume was reduced to only 45 per cent of its amount during bodily rest. He collapsed, and fell off the bicycle.

TABLE 3

Data on the circulation in 4 men of rather poor heart action

The first shows a tachycardia during rest with subnormal stroke volume; the stroke volume becomes normal under exercise. The third line of figures for this man, as compared with the second line, shows second wind with a distinct lowering of the pulse rate. As fatigue develops the stroke volume decreases. This decrease occurs also in the second and third men here shown. The fourth man shows at first a normal adjustment to exercise, and then a development of fatigue, with a pulse of 246 and decreased stroke. He then fell off the bicycle; but was measured again after resting.

BASAL OXYGEN	OXYGEN CONSUMPTION	PULSE	RESPIRATION	R. Q.	CIRCULATION	A-V DIFFERENCE	STROKE VOLUME	STROKE INDEX	REMARKS
0.228	0.349	94	8.14	0.88	6.4	5.95	0.068	0.99	At rest, sitting
	1.44	144	37.0	0.986	17.6	8.2	0.122	1.74	After 3 minutes of exercise
	1.53	126	35.2	0.89	19.3	8.1	0.153	2.19	After 15 minutes of exercise
	2.26	128	36.4	0.60	17.5	12.85	0.137	1.96	After 50 mimutes of exercise
	2.48	150	39.4	0.66	16.6	14.88	0.111	1.59	After 75 minutes of exercise Tired
	1.98	165	40.8	0.79	13.7	14.5	0.083	1.175	After 85 minutes of exercise Very tired
	0.458	128	9.5	0.67	6.9	6.63	0.054	0.771	At rest, sitting 10 minutes' after exercise
0.240	0.554	85	12.2	0.67	10.9	5.1	0.129	1.72	At rest, sitting
	3.48	196	57.3	0.67	19.6	17.7	0.100	1.33	After 5 minutes' exercise
0.237	0.389	74	10.2	0.85	8.9	4.35	0.120	1.6	At rest, sitting
	2.64	188	56.5	0.87	18.7	13.6	0.100	1.37	After 5 minutes' exercise
0.239	0.243	80	5.72	0.79	10.6	2.3	0.133	2.1	At rest, sitting
	2.87	138	50.8	0.722	19.25	14.9	0.142	2.25	After 3 minutes of exercise
	2.78	148	58.2	0.730	18.5	15.0	0.125	1.99	After 14 minutes of exercise
	2.40	246	64.0	0.91	14.2	16.9	0.059	0.86	After 30 minutes of exercise exhausted
	0.591	90	8.58	0.62	7.94	7.9	0.083	1.32	At rest sitting, 14 minutes after exercise
	0.409	88	6.43	0.70	6.51	6.29	0.074	1.18	At rest sitting, 28 minutes after exercise

The influence of tobacco on cardiac efficiency. A closely related form of incoördination between venous return and pulse rate, with consequent restriction of stroke volume, appears to be due to excessive smoking. The

A-V oxygen difference during rest has about the same magnitude for healthy men of all pulse rates, whether smokers or not, or is only slightly higher in those who use tobacco excessively. This approximate constancy of the A-V difference indicates that in all healthy men the circulation during rest is determined chiefly, not by the heart, but by the control exerted upon the volume of the venous return by the oxidative metabolism. This function is not affected by tobacco; but the heart rate is accelerated, and as a necessary consequence (for the arithmetical reason explained previously) the stroke volume is thus decreased.

As an illustration of what may be termed the "tobacco effect" we may take the case of a man with a quite normal circulation of 6 liters per minute and a pulse of 60; the stroke volume is 100 cc. As a result of the excessive use of tobacco his resting pulse is accelerated to 80; but the circulation is still 6 liters. His stroke volume is then only $6000/80 = 75$ cc.; this effect is similar to that which atropine is known to produce. Under moderate exertion and increase of venous return the stroke volume may rise temporarily to about 100 cc. Under intense exertion, however, the behavior of his heart is distinctly less efficient than without the effect of the drug; for if under the exertion the heart rate is 160 beats per minute, instead of 120, as the rate would have been for the same exertion without the drug, the more rapid rate greatly decreases the ease with which the heart carries its load during intense muscular exertion.

In another case a varsity athlete was allowed to break training for a fortnight because of a minor injury. He had improved his opportunities for pleasure so well that the standard exercise on the bicycle induced a pulse of 200. He then returned to training and after another fortnight came to the laboratory to be tested. Under the same exercise his pulse was now 140. Experience in this laboratory shows that in such cases the man when out of condition has a markedly lowered resting alveolar CO_2. He cannot "blow 5.5" (in per cent alveolar CO_2 by the Haldane and Priestley method) after a night of gaiety. The lowered blood alkali thus indicated is probably the factor which underlies the abnormally augmented respiration and acceleration of pulse under exertion. His wind is short.

CONCLUSIONS

Data are here reported by typical examples for the circulation and cardiac efficiency in 50 college students, including men taking little exercise, others taking moderate exercise, and athletes engaging in the most strenuous exertion. The circulation of the non-athletic group approximately doubled in passing from rest to exercise; from the basal state to the exercise it would have been nearly tripled. The circulation of the athletic group tripled in passing from rest to exercise; and the basal circulation would have been quadrupled.

The chief factor determining the minute volume of the circulation is the control exerted by the respiratory metabolism over the venous return, that is, the volume of blood from the venous reservoirs of the body supplied to the right heart.

Exact coördination of heart action and venous return occurs when the pulse rate is such that during bodily rest and moderate exertion these two factors vary proportionally. Under these conditions the stroke volume is uniform in rest and exercise, and the circulation varies in proportion to the pulse. This is the normal relation.

In athletes the pulse rate tends to be much slower and the stroke volume distinctly larger both during rest and exercise than in non-athletes. This slowness of pulse is found to have the advantage of allowing longer diastoles with ample time for the ventricles to relax and fill. As a consequence also the stroke volume in athletes during exertion may be increased considerably, 50 per cent or more, over that during rest, with a corresponding gain in the minute volume of the circulation and its oxygen transporting capacity. The athlete's heart is supernormal.

In "second wind" the pulse and respiration drop from the excessive rates attained at the beginning of the exercise, and for a time thereafter remain at values indicating that the supply of oxygen by the circulation is in balance with the demand. Although the oxygen debt is large it remains uniform. Later as the symptoms of fatigue develop—and this condition comes the sooner the greater the exertion in relation to the man's capacity—the pulse rate rises again. It may reach abnormally rapid rates. The stroke volume of the heart is thereby greatly decreased. Collapse results.

The ill-effects of tobacco on "wind" may be explained by the rapid pulse rates induced by excessive smoking. Owing to the more rapid pulse the stroke volume is decreased during rest, for the venous return and circulation rate are not affected. The ventricles thus lose the habit and ability of making large strokes. During exertion also the stroke volume is therefore smaller, the rate of beat more rapid, and the diastolic relaxation and refilling of the ventricles abbreviated and diminished in comparison to conditions when the man is in training. As the circulation is thus decreased the arterio-venous oxygen difference and the oxygen debt are increased.

The efficiency of the heart is defined as the ratio of the circulation, its volume per minute, to the oxygen requirement of the body. The index of efficiency is the arterio-venous oxygen difference.

Note: Evidence for the accuracy of the ethyl iodide method and significant results from 600 normal and pathological cases have just now been published by Mobitz (14).

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COLLOID PROPERTIES OF THE SURFACE OF THE LIVING CELL

III. Electric Impedance and Reactance of Blood and Muscle to Alternating Currents of 0–1,500,000 Cycles per Second

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The method of electrical resistance by means of the Wheatstone bridge for studying changes in the plasma membrane of irritable tissues which was introduced by the writer (McClendon, 1910) has since been used by a number of workers and the space here is not sufficient to review the literature. Stimulation is accompanied by an increase in permeability indicated by a decrease in resistance.

Höber (1910) introduced the method of high frequency electric currents for measuring the electric resistance of the cell interior. Philippson (1921) made an extensive series of measurements using a modification of Höber's method with improved apparatus and found that a frequency greater than a million cycles per second was necessary in order to approximate the resistance of the interior of the living cell. Since the Wheatstone bridge is perhaps the most accurate method for measuring electric resistance (as well as inductance and capacity) the attempt was made by the writer (McClendon, 1920) to adapt it for the present purposes by increasing the frequency. At that time errors were observed on increasing the frequency to 500,000 cycles per second, and a number of papers have been published since then showing progress in improvement in the technique. It was observed by Fricke as well as the writer that equal ratio arms in the Wheatstone bridge were preferable but variable ratio arms were used except in the latest bridge made by the writer. The difficulty in using equal ratio arms is the difficulty in obtaining standard resistances capable of sufficient variation and yet with low enough reactance. Wire resistances exhibit distributed capacity which increases with increase in resistance. Metallic films have been used but they do not remain constant. Recently the writer has used carbon resistances

made by mixing bakelite with wood flour, pressing into shape and heating to carbonize the wood.

The plasma membrane is a film only a few molecules in thickness on the outer surface of the protoplasm of the living cell. It presents high resistance to the passage of direct electric currents and behaves as the dielectric of a condenser to alternating currents, offering an impedance $Z = -\frac{1}{2\pi fC}$.

If f is the frequency in cycles per second and C the capacity in farads of the condenser formed of the plasma membrane and the conducting solutions on the two sides, Z will be in ohms. Using the Wheatstone bridge, it is theoretically not possible to add ohms due to resistance to ohms due to capacity reactance (though practically a small fraction of the value of one may be added to the other) but resistance must be balanced with resistance and capacity with capacity (or neutralized with inductance). With complex circuits, however, the relation is not so simple, hence the circuits should be as simple as possible. In a previous paper (McClendon, 1926b) difficulties encountered in Wheatstone Bridge measurements at high frequency were pointed out. This paper is an attempt to meet these difficulties.

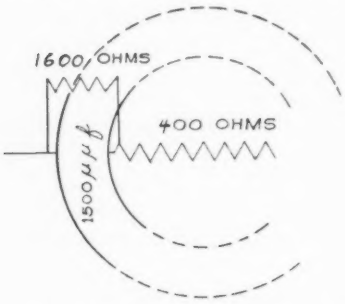

Fig. 1. Diagram showing specific impedance of erythrocyte interior = 400 ohms, impedance of plasma membrane = 1600 ohms and capacity of plasma membrane = 1500 micro-micro-farads.

Measurements on ox erythrocytes. The Wheatstone bridge shown in figure 2 was used for both low and high frequency measurements. With much change of frequency the transformers had to be changed. The 2-stage amplifier could be used to advantage only at or near 1,000 cycles. For high frequency, a heterodyne oscillator was used and the beat-note of 1,000 cycles amplified and heard in the telephone. At audio frequencies the heterodyne was not needed. For part of the work with low frequencies, Vreeland oscillators were used. When these were placed as far as possible (10 meters) from the bridge, their magnetic fields penetrated the copper shielding and disturbed the measurements. The smaller Vreeland oscillator, when placed in a very large iron container ceased to disturb the work, but in attempting to shield the larger oscillator with iron, its frequency changed at a given condenser setting. By moving the oscillator the effects of its magnetic field on the four arms of the bridge were balanced and iron shielding made

unnecessary. Copper shielding was absolutely necessary at high frequency over all parts of the apparatus including the A and B batteries. Partial calibration of the method is given in table 1.

Direct current measurements were made with a Christiansen Ionometer and are therefore not as accurate as the measurements with alternating current.

TABLE 1

Calibration of bridge

Specific impedance of beef blood at 1,000 cycles (25°)

CELL VOLUME	HIGH FREQUENCY BRIDGE	GENERAL RADIO CO. DECADE BRIDGE
per cent		
18.1	100.8	100.3
36.1	147.8	145.8
51.9	198.75	199.1
61.2	260.7	260.0
72.5	352.2	351.0
82.2	497.5	496.6
89.3	658.4	658.2
93.0	1017.8	1014.0
96.2	1164.0	1171.0

TABLE 2

Effects of laking with 110 volt direct current upon the impedance of red corpuscles (25°) (cell volume = 100 per cent)

FREQUENCY IN CYCLES PER SECOND	BEFORE LAKING	AFTER LAKING	TIME OF ELECTROLYSIS
			minutes
1,000	1884.0	535.2	31
1,500,000	322.0	322.0	
1,000	1592.5	641.5	15
1,500,000	410.8	411.7	
1,000	1700.0	638.6	50
1,500,000	485.0	484.1	

In an attempt to determine the impedance (resistance) of the cell interior, ox erythrocytes were packed down by centrifuging in vacuo at 20,000 revolutions per minute, their impedance determined at 1,000 and 1,500,000 cycles, then they were hemolyzed by electrolysis with a 110 volt D. C. current and their impedance determined the second time. The results are shown in table 2. Their impedance at 1,500,000 had changed only within the limit of error of the measurements, whereas their impedance at 1,000 cycles was reduced to $\frac{1}{3}$ its former value. The

TABLE 3

Effect of settling of erythrocytes

CELL VOLUME	IMMEDIATELY AFTER STIRRING	TIME IN MINUTES	AFTER SETTLING	CHANGE WITH SETTLING
Impedance at 1,000 cycles per second at 25°				
				per cent
14.48	97.7	1	96.5	1.23
18.1	102.4	1	101.2	1.17
		2	100.8	1.56
20.85	110.7	1	109.9	1.08
28.7	131.7	1	130.7	0.76
28.96	130.75	1	130.2	0.42
36.1	149.1	1	147.8	0.872
41.7	159.3	1	158.7	0.69
51.9	199.8	1	199.4	0.2
		2	198.75	0.525
59.4	249.9	1	247.7	0.88
61.2	263.2	½	262.7	0.19
		1	261.8	0.532
		2	260.7	0.689
70.7	353.3	1	235.3	0.42
72.5	354.3	½	353.2	0.311
		1	352.2	0.593
82.2	503.5	½	498.2	1.05
		1	497.5	1.19
87.3	667.0	1	663.5	0.53
89.3	659.0	1	658.4	0.091
90.0	872.0	1	865.0	0.80
93.0	1035.0	1	1017.8	1.66
94.7	1164.0	1	1160.3	0.43
96.2	1195.0	½	1172.0	1.925
		2	1167.8	2.275
		5	1164.0	2.595
Impedance at 1,500,000 cycles per second				
28.7	117.7	1½	115.3	2.04
51.9	173.0	1	171.2	1.04
		3	170.3	1.56
		5	170.1	1.68
61.2	196.7	1	194.9	0.92
		2½	194.4	1.17
72.5	245.6	1	241.2	1.79
		2	240.5	2.08
		3	240.15	2.22
82.2	279.9	1	276.7	1.14
		2	276.2	1.32
89.3	297.3	1	296.8	0.168
93.0	324.7	1	323.0	0.96
96.2	337.0	1	334.6	0.71
		2	332.5	1.34

specific impedance after laking seemed to be about 400 ohms but this value was made uncertain by errors in determining the cell constant (which was calculated from its dimensions, and differed in each experiment). In one case it was calibrated with a salt solution but the opening of the cell may have changed it slightly.

In case of mixtures of corpuscles and plasma, settling and orientation of the corpuscles lowers the impedance. Since the determinations could be made very rapidly and verified within a few seconds after stirring (by pressing a rubber bulb without opening the apparatus) the effects of orientation and settling could be studied. Orientation, with the short axis of the erythrocyte perpendicular to the current lines due to passage of the current, was so slight that it could be neglected, but the effects of settling were appreciable, as shown in table 3.

The value of *specific* impedance of ox blood, Z_b, and serum, Z_s, and the inductance, L, placed in series with the conductivity vessel in order to reduce its reactance to zero were tabulated. The conductivity cell constants for 100 per cent cell volume were calculated from the dimensions of the conductivity cells and are not as accurate as that for 14.48–99.9 per cent cell volume. But the ratios of impedance of erythrocytes at 1,000 and at 1,500,000 are not vitiated by incorrect cell constants and the values of the ratios used in constructing figure 3 (4.99 to 7.07) are much greater than the corresponding values calculated from table 2. Apparently the erythrocytes used in the experiments recorded in table 2 were injured at the start to a greater degree than those used in the experiments used in constructing figure 3.

It seems probable that the erythrocyte (in terms of specific values) behaves as the circuit shown in the diagram, figure 1.

As shown graphically in figure 3, the impedance at 1,000 cycles increases rapidly as the cell volume approaches 100 per cent, but the limit cannot be determined with accuracy owing to the fact that centrifuging the blood to remove the serum injures the corpuscles and lowers their impedance. The impedance at 1,500,000 cycles is much less than at 1,000,000 cycles and since in table 2 it is seen that hemolysis does not change the impedance at 1,500,000, one might assume that the impedance at 1,500,000 is the same as at infinite frequency. (This question will be further studied, however, with a more accurate decade Wheatstone bridge with equal ratio arms that has been built for this purpose.)

Measurements on muscle tissue. A Vreeland oscillator giving an alternating electric current of pure sine wave form and 1000 cycles per second was used. The current was cut down by means of a rheostat so as not to stimulate curarized muscle. Frog's muscles were packed in a glass tube having at each end a chamber containing a platinized gold electrode in Ringer's fluid, separated from the muscle by a gold grid. A Wheat-

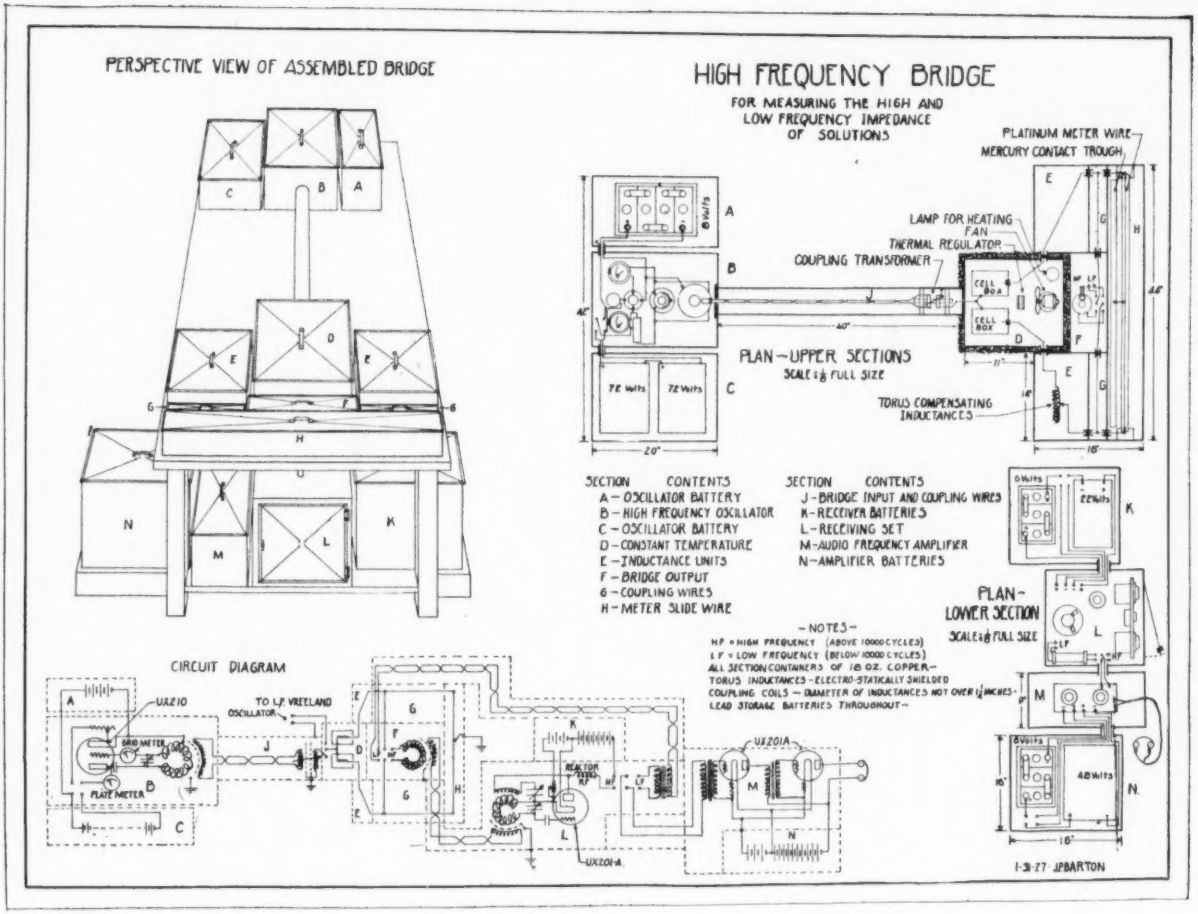
PERSPECTIVE VIEW OF ASSEMBLED BRIDGE
HIGH FREQUENCY BRIDGE
FOR MEASURING THE HIGH AND LOW FREQUENCY IMPEDANCE OF SOLUTIONS
PLATINUM METER WIRE
MERCURY CONTACT TROUGH
LAMP FOR HEATING
FAN
THERMAL REGULATOR
COUPLING TRANSFORMER
CELL BOX
TORUS COMPENSATING INDUCTANCES
PLAN—UPPER SECTIONS
SCALE 1/8 FULL SIZE
PLAN—LOWER SECTION
SCALE 1/8 FULL SIZE
SECTION CONTENTS
A—OSCILLATOR BATTERY
B—HIGH FREQUENCY OSCILLATOR
C—OSCILLATOR BATTERY
D—CONSTANT TEMPERATURE
E—INDUCTANCE UNITS
F—BRIDGE OUTPUT
G—COUPLING WIRES
H—METER SLIDE WIRE
SECTION CONTENTS
J—BRIDGE INPUT AND COUPLING WIRES
K—RECEIVER BATTERIES
L—RECEIVING SET
M—AUDIO FREQUENCY AMPLIFIER
N—AMPLIFIER BATTERIES
—NOTES—
HF = HIGH FREQUENCY (ABOVE 10000 CYCLES)
LF = LOW FREQUENCY (BELOW 10000 CYCLES)
ALL SECTION CONTAINERS OF 16 OZ. COPPER—
TORUS INDUCTANCES—ELECTRO-STATICALLY SHIELDED
COUPLING COILS — DIAMETER OF INDUCTANCES NOT OVER 1½ INCHES—
LEAD STORAGE BATTERIES THROUGHOUT—
CIRCUIT DIAGRAM
UX210
TO L.F. VREELAND OSCILLATOR
GRID METER
PLATE METER
REACTOR
UX201A
UX201A
1-31-27 JPBARTON

Fig. 2

stone bridge having negligible inductance was used. The capacity of the conductivity cell and muscle fibers was balanced by placing a variable condenser in parallel with the standard resistance. A tuned telephone was used as null-instrument. Since a tone-silence cannot be obtained unless the capacities (as well as resistances) are balanced, the method enabled us to estimate the capacity of the conductivity vessel and muscle, and knowing the capacity of the vessel filled with Ringer's fluid, the capacity of the muscle could be calculated. Whereas the values for impedance were found to be accurate, the values for capacity were not corrected for distributed capacity and dielectric loss and are not given here since more correct values will be published later.

An attempt was made to determine the difference in impedance of the stimulated and unstimulated muscle. By cutting out resistance at the oscillator the current could be caused to stimulate the muscle but it was

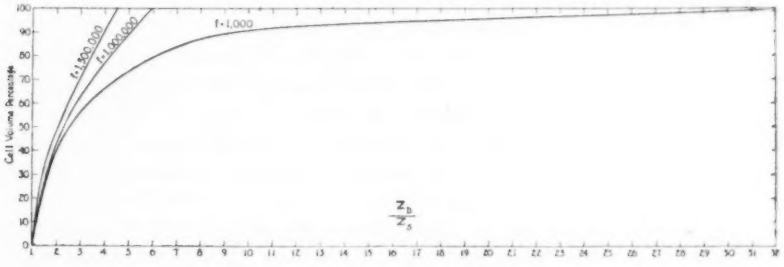

Fig. 3. Graph showing relation of cell volume percentage to impedance of ox blood (Zb) on the basis of the impedance of serum (Zs) as unity, at 1000 cycles(f = 1000) 1,000,000 cycles (f = 1,000,000) and 1,500,000 cycles (f = 1,500,000).

difficult to tell when stimulation occurred with the muscles packed in the tube.

To obviate this difficulty, curarized turtle's muscle was placed between two bright platinum discs that were rigidly fixed and that slightly squeezed the muscle. On stimulating the fresh muscle, its impedance decreased to $\frac{1}{4}$, but the decrease was less as the muscle became fatigued. The experiments were done in air of constant temperature ($20^\circ \pm 0.1^\circ$). The backs and edges of the platinum discs were paraffined so as to limit conduction to the fronts of the discs but the contraction of the muscle changed the shape of the projecting edges.

The impedance of the resting muscle increased with time. The standard resistance was not changed and the ratio of the ratio arms is given in the body of the table below. In one experiment the impedance of the resting muscle, measured immediately after stimulation, was 0.87 and 15 minutes later was 0.96, showing slow recovery.

Successive measurements of impedance on curarized turtle muscle

	PHYSIOLOGICAL STATE						
	Rest	Stimulation	Rest	Stimulation	Rest	Stimulation	Rest
Period	1	2	3	4	5	6	7
1st muscle	1.145	0.398	0.628	0.395	0.522	0.357	0.471
2nd muscle	2.770	0.693	1.590	0.693	1.350		
3rd muscle	2.300	0.640	0.962	0.555	0.612		

The curare made the muscle more difficult to stimulate with currents of 0.0005 second duration since it lengthened the chronaxie. McClendon, Fetterly and Hinniker have used the Vreeland oscillator to determine the duration (under certain conditions) of the minimum effective stimulus of muscle and found it to be increased by curare, confirming Lapique.

SUMMARY

A new, high-frequency-Wheatstone-bridge has been built, with heterodyne detection and 2 stage amplification and all parts, including the A and B batteries, shielded in copper (fig. 2).

The specific impedance of erythrocytes at 1,500,000 cycles per second seems to be about the same as of the erythrocyte interiors and to be about 400 ohms or roughly that of a 0.02 *N* or 0.1 per cent NaCl solution.

The electric resistance of the erythrocyte surface is very high but probably much less than that of Valonia. It seems improbable that Osterhout's conclusions as to the impermeability of Valonia to ions can be applied to ox erythrocytes.

The impedance of muscle tissue to currents of 1000 cycles per second is much greater at rest than during stimulation, confirming the earlier measurements made by the writer (McClendon, 1912). Fatigue is characterized by decrease in impedance of resting muscle and recovery by increase in impedance.

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THE MONOPHASIC ACTION POTENTIAL CURVE OF TORTOISE VENTRICULAR MUSCLE

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The recorded electrocardiogram is always the expression of the electrical balance existing between two effective galvanometer (or electrometer) leads from the reacting tissue. It is, therefore, desirable to obtain an idea of the electrical changes occurring at a single point in a heart preparation excited by an artificial stimulus, the time relations of which can be accurately known. The present paper deals with an attempt to obtain such curves. The results of this attempt lead to the conclusion that, in strip preparations, the recorded electrocardiogram is due to the summation of a series of local monophasic electrograms which may be reconstructed from recorded electrograms. The monophasic electrogram shows a prolonged period of negativity which develops with the excitation of the tissue, continues through the period of contraction and subsides during the period of relaxation of the muscle.

The results to be discussed in this paper were obtained by using tortoise ventricular strips. The strip preparation was selected in order to make possible the use of cardiac tissue in a form giving an approach to linear fiber arrangement. Furthermore, in order to avoid the possibility of complications due to conduction of the impulse through the strip, arrangements were made to lead to the galvanometer from the site of stimulation. This procedure has been followed previously by Erlanger, Gasser and Bishop (1924) in a study of nerve action potentials. It has not, so far as I am aware, been reported as having been used in a study of muscular tissue.

Although the present paper deals only with the results of studies made upon tortoise ventricular strips, it may be stated that corresponding results have been obtained from auricular and ventricular tissue of both cold-blooded and mammalian hearts.

APPARATUS AND METHOD. The electrical changes studied were recorded by use of a capillary electrometer. The successful operation of this instrument requires some patience and most records obtained with the old style, fine-bored sensitive capillaries require correction before true curves

are to be had. In most laboratories the capillary electrometer has been replaced by the string galvanometer. The capillary electrometer has an advantage over the string galvanometer, however, in the relative indestructibility of its sensitive mechanism. In the course of the work to be described below, it was desired to stimulate the strips by induction shocks which were shunted into the recording instrument. A slight unbalance of the stimulating circuit would result in setting up an excessive potential difference across the instrument and such a technique promised a high mortality of strings if the Einthoven galvanometer were to be used. For this reason, it seemed eminently desirable to attempt the use of a capillary electrometer.

Preliminary work revealed the facts that an electrometer having a capillary with nearly parallel walls and a bore of about 0.2 mm. at the working region gave a fairly fast working but rather insensitive electrometer. It was a simple matter to arrange such an electrometer with a single stage of amplification. With such a combination, it was possible to record electrograms which, for qualitative purposes, could usually be studied without correction of the records. As was shown by Broemser (1922) the aperiodicity of such a capillary is less than that of one with a greater degree of taper but the slight periodicity of the capillary in use has not proved a serious handicap in our work. The construction of the electrometer which was employed in these experiments has been described elsewhere (Gilson and Bishop, 1927) and was such as to furnish a convenient form of capillary electrometer which should be nearly free from vibrations of the capillary meniscus as a result of passing traffic outside the laboratory building. The type of electrometer now in use has made it possible to work during the passage of ordinary traffic along the street, but severe jarring of the building still causes slight irregularties in the recorded curves.

As arranged for recording, the capillary is illuminated by the light from an arc lamp, the image of the meniscus being projected by a microscope which carries a 16 mm. objective. The slit of the recording camera is placed at 130 cm. from the capillary.

The electrical circuit used in these experiments is diagrammed in figure 1 *A*. Two calomel electrodes carrying worsted wicks soaked with Ringer's solution are attached to a ventricular strip. The wicks are fastened in place by stitching them under the epicardium or by tying to the epicardium with silk thread. One electrode is grounded. The other electrode leads to the grid of a thermionic vacuum tube amplifier. The amplifier circuit employs the Wheatstone bridge construction used by Goode (1925), the variable resistance, *Rv*, being made equal to the resistance at zero input, of the plate circuit, *Rp*, and the B-battery separated into two parts, E_1 and E_2, which have equal voltages. The point at which the output

condenser, C, is placed is therefore at zero potential with respect to the ground except when changes in the grid potentia cause corresponding changes in the potential at the plate. The use of such a circuit prevents the continuous drift of the capillary meniscus which is present when the usual B-battery arrangement is used with an output condenser which is not extremely well insulated.

When a source of potential difference is applied directly across the capillary (without an amplifier) the meniscus shows a rapid excursion through a distance nearly proportional to the applied voltage (fig. 2, curves A and B). The displacement of the meniscus is maintained until the potential difference across the capillary is removed, when there follows a quick return

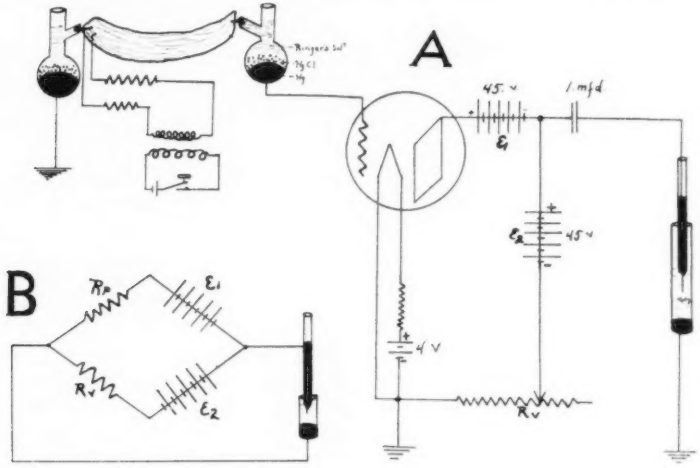

Fig. 1. A. Wiring diagram of circuit used. B. Diagram illustrating Wheatstone bridge arrangement used in B battery circuit of amplifier.

to the base line. If the amplifier is placed in the circuit (fig. 2 C), and a source of potential difference applied to the grid circuit, there is a movement of the meniscus away from the base line, followed by a slight, nearly logarithmic drop to a new level due to the slow discharge of output condenser under the continued unbalance of the plate circuit. The form of this curve may be determined for different voltages and correction of records made in a manner similar to that used in correcting records made directly with the capillary electrometer. With an output condenser of the capacity used (1 mfd.) the falling off due to the discharge of the condenser comes to half value in about 0.3 second. This is sufficiently slow to introduce no serious qualitative error into most of the curves recorded.

In order to obtain an effective electrometer lead from the point on the tissue at which the stimulus is applied, the ground electrode is made the cathodal electrode for the stimulating break induction shocks from the secondary of a Harvard inductorium. The anodal terminal of the secondary is usually connected to a fine tinsel wire attached to the heart three to five millimeters from the cathode. Variable resistances placed in the secondary circuit make it possible to reduce the recorded escape of the shock to a minimum. The grid electrode is attached to the strip at any desirable distance from the ground electrode.

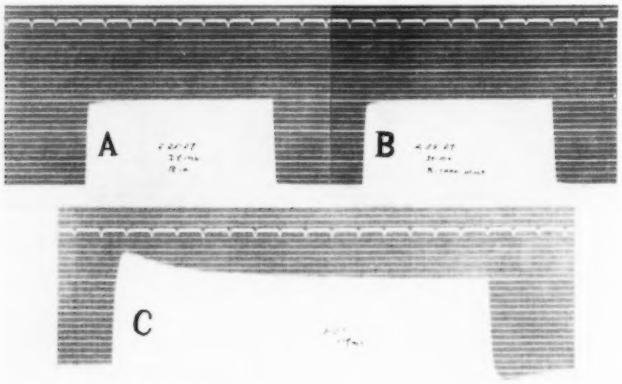

Fig. 2. "Normal curves" showing responses of meniscus to instantaneously applied difference of potential. Time record shows $\frac{1}{5}$ second intervals. *A*, 34 mv. direct to capillary. No resistance added to circuit; *B*, 34 mv. direct to capillary. 1,000 ohms in series to prevent overthrow of meniscus; *C*, 8.4 mv. applied to grid circuit of amplifier placed in capillary circuit.

The heart strip preparations are cut from the ventricle of the tortoise Pseudemys concinna and range in length up to about 30 mm. The preparations are arranged in a Harvard moist chamber. Injury is usually produced, when desired, by crushing with heavy forceps. The mechanical records are obtained by attaching a light heart lever to the strip at the point of attachment of the grid electrode, the strip being suspended in such cases by the ground electrode. The lever is placed in front of the slit of the recording camera and the movements of the lever, the capillary meniscus, and the point of a Jaquet chronograph marking $\frac{1}{5}$ second intervals are photographed simultaneously.

Description of records. Rather than attempt the use of terms which shall specifically apply to all of the possible variations observed in the

electrograms recorded from strip preparations, the following terminology will be followed:

The initial complex of short duration is referred to as the R-wave, or if there be marked diphasicity *of the initial complex*, as R- and S-waves. (A Q-wave is not developed by a preparation with one electrometer lead from the site of stimulation.) The final complex is referred to as the T-wave. This may be monophasic or diphasic in form. In practically all records from strip preparations, the last part of the T-wave is of sign opposite to that of the R-wave. If the grid electrode be on subnormal tissue, however, the first and most striking part of the final complex (T-wave) is positive in direction.

Waves occurring between the R-S and T-waves have been largely ignored in the discussion to follow. They are present in the majority of strip records and are due to morphological irregularities of the preparation and similar extrinsic effects, as well as to differences in the condition of the tissue under the two electrodes.

A purely diphasic record (fig. 3 *A*) may be obtained when a fairly long strip is arranged with the lead-off electrodes well separated and on regions of the tissue in similar physiological conditions. Such a record shows the escape of the stimulating shock as a low sharp spike. Rising almost immediately out of the shock is the R-wave which attains a maximum and falls back toward the base line. This is followed by a nearly flat portion of the curve, the R-T interval, during which the curve runs close to the base line. At about the time when the peak of the mechanical contraction has been reached, there commences a T-wave which is of opposite sign from the R-wave. The T-wave attains a maximum and returns to the base line during the latter part of the period of mechanical relaxation. If the distance between the electrodes be made less, the duration of the R-wave decreases as does the time of the rising phase of the T-wave from leads on fresh uninjured tissue. The R-T interval is little changed, however, and the general shape of the curve remains like that of figure 3 *A*. When an injury occurs to the tissue under one of the electrodes, the curve immediately assumes a different form. Such a record as that shown in figure 3 *B* is obtained under these conditions.

The strip, from which figure 3 *A* was obtained immediately after suspension on the electrodes, was stimulated every few seconds for nearly an hour. At the end of this time, lead A (the ground and stimulating electrode) was removed from the end of the strip and carefully attached to the base of the frenum. Lead B remained at the other end of the strip, close to the cut and dying edge of the strip. One electrode was then on relatively normal tissue, the other on tissue which, if not directly injured itself was at least subject to the effect of injury products from the region around it. The resulting record is shown as figure 3 *B*. The R-wave rises along the

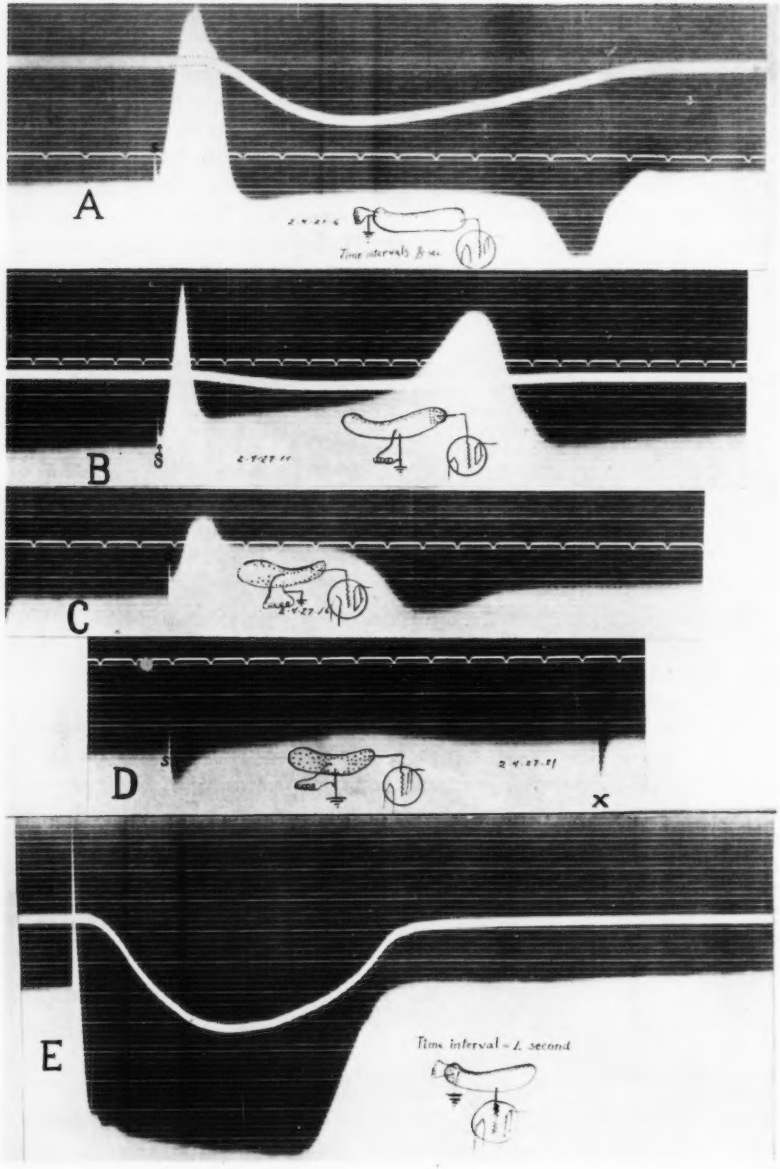

Fig. 3. Capillary electrometer records obtained from ventricular strips. Leads as shown in diagrams, stippling indicates known regions of injury. Time records show $\frac{1}{5}$ second intervals except in *E* where the time intervals are of 1 second duration; *s*, stimulating shock; *x*, an ineffective make shock.

same curve as that of the R-wave in figure 3 A, but it is more quickly brought back to a level, in this case, somewhat above the base line. The T-wave appears chiefly as a positive wave, that is, having the same direction as the R-wave. Further injury, killing back from the grid lead toward the site of stimulation gave records of which figures 3 *C* and 3 *D* are typical. Record 3 *C* was made from the same strip as the foregoing after crushing to within 5 mm. of the ground electrode. The appearance of the very slight contraction following this treatment indicated that only two or three millimeters of tissue remained active. More thorough crushing after which contraction was barely perceptible resulted in the recorded action potential shown as figure 3 *D*. Starting with a fresh preparation, with leads placed on one end and at the middle of the strip, a highly diphasic record was obtained. When, however, the tissue near the ground electrode was crushed so as to leave only a very narow band for conduction for five or six millimeters away from the electrode the grid lead being on fresh tissue, the record reproduced as figure 3 *E* was obtained. In this case, the effective potential at the ground lead was reduced not only by the effects of injury to the tissue but also by the shunting produced by the dead tissue surrounding the tongue of living muscle on which the ground lead was placed. Such a record appears nearly monophasic. (This record was obtained with the stimulating electrodes placed one on either side of the ground electrode. The record of the shock is scarcely visible.) The R-wave from the region stimulated is seen as a spike of short duration. The plateau which follows is of negative sign. As the correction of the curve introduces a somewhat different form, the corrected curve is shown in figure 4 *C*, curve *1*.

The three general types of curve which may be obtained from strip preparations under various conditions may be summarised as follows.

1. Equal effects from the two electrodes. This type is best seen when each electrode is placed on normal tissue. The curve shows an R-wave, an R-T interval during which the curve follows very close to the base line, and a negative T-wave.

2. Slight predominance at one electrode. As when the proximal electrode is on normal tissue, the distal electrode on subnormal tissue. The curve shows an R-wave, sometimes an S-wave, the R-T interval showing an upward slope of the curve, and a T-wave chiefly positive. When the distal lead predominates instead of the proximal there is a pronounced S-wave and a T-wave chiefly downward.

3. Great predominance at one electrode. One electrode on normal tissue, the record being taken immediately after killing the tissue under the other electrode. The limit which this type of curve approaches is a record in which the R- or S-wave fuses with the T-wave giving a continuous nearly monophasic record.

ANALYSIS OF RECORDS. By leading from the site of stimulation, direct evidence is obtained for the prompt rise of the action potential following stimulation, a fact long accepted on the basis of indirect measurements.

Furthermore, regardless of the various theories of muscular response and of the electromyogram, certain frequently neglected points must be considered in the interpretation of any electrocardiogram. These include the following:

1. The electrogram represents changes in potential between the two

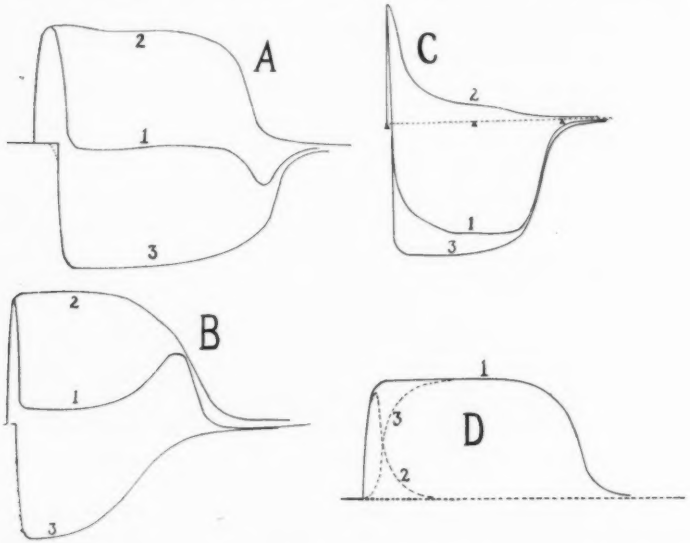

Fig. 4. Reconstructions of monophasic action potential curves. *A* is taken from record 3*A*, *B* from record 3*B*, *C* from record 3*E*. In *A*, *B* and *C*, curve *1* is the corrected capillary curve, curves *2* and *3* respectively are the reconstructed m.a.p. curves from ground and grid leads. In *D*, curve *1* is the m.a.p. curve of normal tissue, curves *2* and *3* the hypothetical curves indicating the electrical changes of the excitation and contraction processes respectively.

effective leads to the galvanometer. Any indication of the direction of impulse movement is subordinate to this condition.

2. With small electrodes attached directly to the heart, the effective leads approach but do not attain to point leads. With leads from the pericardial fluid or from the outside of the body, the effective leads become so diffuse as to include most of the outer surface of the heart. Such diffuse leads do not give accurate representation of the conditions at any particular small region in the heart.

3. The galvanometer circuit may be shunted by the fluid bathing the surface of the heart preparation, reducing the height of the recorded electrocardiogram. This factor becomes of increasing importance as the strip becomes shorter or the heart smaller.

4. Recorded action potential changes do not necessarily arise immediately under the lead-off electrodes. There may be marked extrinsic effects.

5. Immediately after injury near or under one of the electrodes, the records show an approach to monophasicity. Within a few minutes, however, the injury effect decreases. The killed tissue then acts as a diffuse effective lead for the living tissue upon which it borders. The effect upon the recorded action potential curve of the extent of the killed tissue (in nerve) is discussed by Bishop, Erlanger and Gasser.

6. Under the effects of severe fatigue or injury, heart tissue shows an early depression of the plateau of the monophasic action potential curve. There are then produced marked irregularities in the diphasic action potential curves.

If the above facts are borne in mind the analysis of a purely diphasic record of type 1 offers, perhaps, the easiest approach to a reconstruction of the curve of the electrical potential changes occurring at a single point in the heart strip. With a 30 mm. strip, the record obtained is such as that shown in figure 3 *A*. With progressive shortening of the distance between leads, the duration of the R-wave becomes proportionately less, the T-wave tends to become lower and to reach its maximum more quickly, but the duration of the entire electrical reaction shows little if any decrease. Such a condition would occur if the action potential curve which would be led (hypothetically) from a single point on the tissue had the monophasic form shown in figure 4 *A*, curve *2* Assuming this to be the case, the following interpretation of the record is possible. Following the stimulus, there occurs the rapid change of condition at the site of stimulation which gives the rise of the R-wave. Conduction of the impulse along the strip occurs at the rate of about 125 mm. per second (Meek and Eyster, 1912). At this rate, in a 30 mm. strip, excitation occurs at the distal end, *B*, 0.024 second later. At that time, the R-wave has commenced its return to the R-T plateau due to the development of negativity at *B* which becomes equal to that at *A*. Continued equal negativity at both ends of the strip would cause a maintained zero potential difference between the two leads and the record would continue along the base line. If the subsidence of the period of negativity at *A* commenced about 1.2 second after the time of stimulation, and if the same process occurred 0.024 second later at *B*, we should find a negative *T*-wave followed by a slow return toward the base line. The base line would be reached when *B* had completely recovered.

By such a process we can obtain the theoretical monophasic action curves from the points *A* and *B* whose sum gives the diphasic electrogram as recorded.

It is not possible, however, to account for a curve of the form of figure 3 *B* by the summation of two similar monophasic curves of the form shown in figure 4 *A*. The monophasic electrogram from a point on the strip which has been affected by severe fatigue or slight injury shows an initial rise of negativity nearly the same as that in normal tissue. The normal plateau is either reduced in duration or is lacking. That is, instead of maintaining a prolonged high degree of negativity during contraction followed by a rather sharp drop of the curve during the return toward the base line, injured tissue gives a monophasic electrogram showing a much earlier start of the drop but with a much more gradual progress of the curve toward the base line. Figure 4 *B* shows the reconstruction of the monophasic electrograms from the two lead-off points which would give figure 3 *B*, the assumption being made that the proximal lead was on normal tissue giving a monophasic electrogram of the form indicated in figure 4 *A*.

Discussion. The supposition that the electrogram from a single point on cardiac tissue indicated a single continued state of negativity is not a new one. A very close approximation to the diagrammatic monophasic electrocardiogram given in this paper was described by Burdon-Sanderson and Page (1880) who used the Bernstein differential rheotome in their experimental work. Samojloff (1914) has used a similar monophasic curve in the interpretation of experimental changes in the electrocardiogram. A number of authors have described the persistence of the electrical change into or during the period of systole in both ventricular and auricular muscle. All curves that we have been able to find in the literature, however, showed more or less diphasicity, even though the curves were obtained by summing or subtracting other curves. This diphasicity is usually due to extrinsic effects acting upon the circuit or to acceptance of the tradition that a galvanometer lead placed on killed tissue may be regarded as entirely indifferent. Such attempts at subtraction of curves have been made by Joly (1913) and by Drury and Brow (1926).

Lewis, Meakins and White (1914) found that even with leads placed directly upon cardiac tissue, potential changes at points not under the electrodes might have considerable effect upon the form of the recorded curve. Similar conditions have been shown to hold in the study of nerve by Bishop, Erlanger and Gasser (1926), and in skeletal muscle (unpublished observation made in collaboration with Doctor Bishop).[1]

[1] It was originally intended that the paper by Dr. Bishop and myself on the action potential of skeletal muscle (This journal, vol. lxxxii, p. 478) should follow the present paper. Certain references in the joint paper may be somewhat clarified if the reader bears this fact in mind.

Lewis, Meakins and White used the terms *intrinsic* and *extrinsic*, the former effects beng those due to changes occurring directly beneath the electrodes, the latter those changes occurring elsewhed in the preparation but affecting the recording mechanism. The extrinsic effects are always present in any preparation and may modify the recorded changes considerably. In a heart strip of fairly symmetrical nature, connected to the electrometer by direct leads, the extrinsic effects tend to balance out and the record may be assumed to approach a diphasic record indicating the electrical balance existing between the tissue at the two lead-off electrodes.

Einthoven defends the theory of the electrocardiogram as representing an electrical change concomitant with the mechanical contraction. This view may be regarded as opposed to that expressed by A. Hoffmann that the electrocardiogram represents two processes, the R-wave being due to the rise of the excitatory process, the T-wave to the rise of a contraction process. To consider the T-wave as a discrete entity does not seem justifiable. It is possible that if there be a separability of the electrical responses of excitation and contraction, they would give curves of the form indicated in figure 4 *D*.

The "theory of electrical doublets" recently proposed by Craib (1927) offers an interesting approach to the study of unit electrical activity in reacting muscle. Craib's interpretation of the waves recorded from heart strips does not, however, seem to be a correct one. Records, from heart strips, which approach a monophasic form do not show discrete T-waves in a direction to indicate electrical positivity with respect to resting tissue. Further, the R-wave of a simple strip preparation (stimulated at one end and led to the galvanometer from two points along the strip) is not preceded by a Q-wave. The nature of Craib's strip preparation postulates the presence of considerable injured tissue, particularly at the apical end of the strip, and all of the records which he publishes may be more readily explained on the basis of the character of the monophasic action potential curves which has been developed above.

The DeMeyer phenomenon, a recorded change of potential during stretching of muscular tissue, is not causally related to the negativity maintained during muscular contraction. Experiments performed upon the stretching of strips under the conditions of the experiments reported above showed a recorded change of potential with stretching of less than 5 per cent the height of the recorded electrograms. In such experiments, strips were attached to the electrodes and to a writing lever. In many cases no potential change was observed until the stretching was sufficient to stimulate the strip and set up a complete contraction cycle. In some cases where one electrode was attached to an extensive region of freshly killed tissue, stretching almost to the point of stimulation caused a movement of the meniscus shadow through a distance of about 3 millimeters, a distance corresponding to a potential change of about 0.6 mv. between the

electrodes. Under no circumstances could a greater potential difference be produced by a nonstimulating stretch. The T-wave of a diphasic record from a thoroughly moist ventricular strip preparation shows an effective potential difference between the electrodes of about 15 mv.

The above experiments also served as controls upon the possibility of recorded potential changes as a result of movement of the electrodes upon the preparation. Such manipulation of the preparation after attachment of the electrodes caused no essential changes in the records.

Numerous authors have shown that special tissue differentiation is unnecessary for the production of the polyphasic electrocardiogram. Such records have been obtained from the sinus venosus, the auricle, ventricle, and bulbus aortae, from preparations with leads on intact hearts and from excised strips of tissue. The experiments reported above and others upon auricular tissue and intact hearts show how easily changes in tissue condition or the position of the effective leads influence the form of the recorded electrogram. The analysis of such records into summating monophasic curves offers a means of obtaining increased accuracy in the interpretation of records.

The nature of the error in reconstructing the monophasic action potential curves is such that the time relations between the end of contraction and the falling off of the local negativity have not been determined with great accuracy. Experiments now in progress indicate the probability that the qualitative curves given in this paper may be essentially verified in a quantitative manner.

SUMMARY AND CONCLUSIONS

1. The use of a capillary electrometer nearly free from movements of the meniscus due to external vibration furnishes a valuable instrument for the study of the action potential changes of cardiac tissue. The relative insensitiveness of the electrometer used offers no serious drawback when the action potential changes are amplified by a one panel thermionic vacuum tube amplifier.

2. By proper balance of the stimulating circuit to reduce escape of the shock, it is possible to lead off the action potential changes from the point stimulated and one other point on the preparation.

3. Such records obtained with one lead at the site of stimulation are always of a diphasic nature, showing the relative potential changes occurring under the two effective lead-off electrodes.

4. Direct evidence is obtained for the prompt rise of the action potential following stimulation of the cardiac tissue.

5. An approach to a monophasic record may be obtained by the use of a strip with fresh injury under one electrode.

6. From diphasic electrograms recorded from heart strips, it has been possible to reconstruct a monophasic action potential curve.

7. The monophasic action potential curve shows a rise of negativity immediately after excitation of the tissue and a continuance of the negativity through the period of contraction. Return to resting potential occurs during the relaxation of the muscle.

8. Monophasic action potential curves are not of invariable form. The return toward the base line commences earlier in injured or fatigued tissue.

9. The recorded electrocardiogram may be regarded as the algebraic sum of a series of such monophasic action potential changes taking place along the strip and acting upon the lead-off electrodes. The R- and T-wave are the early and late expressions of this summation.

10. Monophasic action potential curves, though showing changes related to the periods of contraction and relaxation, are not due to "deformation currents" consequent to the shortening of the strip nor to movement of the electrodes upon the surface of the preparation.

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HISTAMINE AND SALIVARY SECRETION[1]

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Various workers (1), (2), (3), have shown that histamine stimulates a flow of saliva. This effect was produced in a whole animal with the secretory salivary nerves intact, as well as after section of the chorda tympani. Atropine paralyzed the action of histamine. Therefore, the current view is that histamine acts in the same way as pilocarpine on the nervous mechanism of the gland. The study of the effect of histamine on the salivary glands has a twofold interest; first, because a detailed investigation of the action of this substance on the salivary glands has never been performed, and secondly, it is highly desirable that an analysis of the action of histamine on the digestive glands in general be carried out. The submaxillary gland was chosen as a suitable organ for such a study.

METHODS. Cats and dogs were used for the experiments. In most cases they were anesthetized with a chloroform-ether mixture followed by an intravenous injection of chloralose 0.1 gram per kilogram of body weight. Some experiments were performed on decerebrate cats only under ether anesthesia. The chorda and sympathetic nerves were severed, a cannula was inserted in the duct of the submaxillary gland and connected to an electric drop recorder. The drops registered by this recorder were very small, 56 being required to make 1 cc. In some experiments the blood flow was also measured by Maevsky's method, which is described in another paper (4) or by Gesell's bloodless method. Artificial respiration was introduced at the beginning of all experiments.

The doses of histamine phosphate, from 0.25 to 0.5 mgm. for cats and from 1 to 2 mgm. for dogs, were injected into the femoral vein, followed by 2 cc. of saline to wash the cannula.

General action of histamine on the submaxillary gland. Because some of my early experiments performed on chloralosed cats were not in accordance with the view mentioned above, an investigation was necessary in order to determine the effect of the presence or absence of various narcotics on the action of histamine.

A large number of experiments was carried out on cats and dogs anesthetized with chloralose, in which the gland was denervated before the experiment. In over 50 per cent of the experiments on cats histamine,

[1] This work was carried on under a grant given by the National Research Council of Canada.

whether injected intravenously, subcutaneously or intramuscularly, had no effect in causing a flow of saliva. In the remainder of the experiments histamine provoked a slight secretion varying from one to three drops. Similar results were observed with dogs, half of the experiments giving a negative effect, while the others produced a secretion ranging from 1 to 12 drops.

Other experiments were performed on cats with only ether and chloroform anesthesia, to determine if chloralose was responsible for the variable effect obtained above with histamine. It was found that these two narcotics gave the same results as chloralose, and did not increase the effect of histamine on the gland.

To remove the influence of anesthetics entirely, a few experiments were carried out on decerebrate cats. The results in all cases were positive, although a rather meagre secretion was obtained. Yet the effect of histamine seemed to be more pronounced in the absence of anesthetics.

As the previous experiments were done with a denervated gland, the next step was to find out whether or not the integrity of the chorda tympani and sympathetic altered the action of histamine. In attempting to determine this point two types of experiment were performed: one, on an intact gland under chloralose, the nerves being severed later during the experiment without altering the effect of histamine; another, in which cannulae were inserted in the ducts of both glands, one of which was denervated and the other intact. From the results of both classes it is evident that the action of histamine is independent of the integrity of the extrinsic nerves.

The effect on blood pressure does not vary markedly in the different types of experiments, histamine causing a marked fall which is sometimes preceded by a slight transitory rise. As repeated injections of histamine are given their effect on the blood pressure gradually becomes less. This diminution of the vascular reaction to histamine in many experiments runs parallel with the decrease of the secretory effect; the gland seems to be poisoned by the drug and does not react as in the beginning of the experiment.

The intravenous injection of histamine produces a long-continued acceleration of the blood flow through the gland. In the dog this may be followed later by a diminution in the rate of flow. The increase of the blood flow takes place in spite of a marked fall in blood pressure, and it is usually not markedly affected by successive doses of histamine. The conditions of the blood flow are fully discussed in another paper (4).

These preliminary experiments show that the salivary flow provoked by histamine can not be looked upon as a secretion analogous to that produced by other drugs, e.g., pilocarpine, which acts on the parasympathetic, or adrenalin, acting on the sympathetic nervous system. The

results of the analysis of the action of histamine on the submaxillary gland comprise the remainder of this paper.

An analysis of the action of histamine. The action of histamine was investigated from two aspects; as an agent acting on the secretory elements of the gland, and as a drug which may affect the contractile elements of the organ.

In the gastric glands, histamine probably acts on the secretory cells because its action is not abolished by atropine. It may have a similar effect on the salivary cells. Furthermore, we know histamine causes smooth muscle to contract, and there seems to be evidence for the existence of contractile elements, both around the ducts and enclosing the acini. Therefore, histamine may have a mechanical effect pressing out saliva in addition to a secretory action. From these considerations it was of interest to investigate the action of histamine both on the nervo-glandular structure and on the contractile elements of the salivary gland.

Does histamine act on the nervo-glandular elements of the submaxillary gland and influence the subsequent effect of nerve stimulation? The following method was employed to study the action of histamine, on the response of the gland to excitation of the secretory nerves. The chorda was stimulated for a certain period at intervals of 12 to 14 minutes, to avoid the phenomenon of augmented secretion, so that the normal rate of secretion could be determined. After 12 minutes histamine was injected, and as soon as the blood pressure had approximated its original level, the chorda was stimulated again. The following figures are typical examples of the results obtained throughout this group of experiments.

Experiment 1. Cat. Chloralose.

	SECRETION PER MINUTE	BLOOD PRESSURE
	drops	*mm. Hg*
1 hour 46 minutes:		
Chorda, coil 13, stimulated for 15 seconds	20	168
1 hour 58 minutes:		
Chorda, coil 13, stimulated for 15 seconds	19	158
2 hours 9 minutes:		
Histamine, $\frac{1}{4}$ mgm	1	74–130
2 hours 12 minutes:		
Chorda, coil 13, stimulated for 15 seconds	18	130
2 hours 24 minutes:		
Chorda, coil 13, stimulated for 15 seconds	18	114

It is evident from these figures that the chorda secretion is not increased after histamine. As similar experiments with the sympathetic also gave negative results, it may be concluded that histamine does not increase

the excitability of the gland, so that a subsequent stimulation of either of the secretory nerves will produce an augmented effect.

Influence of histamine on pilocarpine secretion. As such a slight reaction was observed from histamine on the resting gland, it was decided to investigate its effect during activity of the organ. Pilocarpine was first given in a dose sufficient to provoke a good secretion during which histamine was injected. Many experiments were done on cats and dogs of which a typical one is quoted below.

Experiment 2. Cat. Chloralose.

	SECRETION PER MINUTE	BLOOD FLOW	BLOOD PRESSURE
10 hours 30 minutes:			
1 minute before histamine	0	20	138
1 minute after $\frac{1}{4}$ mgm. histamine	0	48	84–102
2 minutes after $\frac{1}{4}$ mgm. histamine	0	38	102
10 hours 43 minutes:			
1 minute before pilocarpine	0	26	62
1 minute after $\frac{1}{4}$ mgm. pilocarpine	13	26	44–64
2 minutes after $\frac{1}{4}$ mgm. histamine	19	48	64
10 hours 46 minutes:			
1 minute before histamine	24	48	68
1 minute after $\frac{1}{4}$ mgm. histamine	3	20	50–62
2 minutes after $\frac{1}{4}$ mgm. histamine	16	38	62

A marked inhibition in the secretion is the usual effect of histamine after pilocarpine in the cat. This inhibition is not dependent on the blood pressure, as the fall after pilocarpine may be as great as that after histamine (see fig. 1). It is interesting to note that in a few instances with cats, histamine produced an acceleration of the pilocarpine secretion, but this was observed when the effect of pilocarpine was wearing off. Different results were obtained in the dog, histamine usually giving an acceleration and an inhibition only after large doses of pilocarpine.

An analysis of this peculiar phenomenon showed that the inhibition in secretion was due to a parallel inhibition of the blood flow through the gland, which is exemplified in the above experiment. As histamine by itself usually produces an acceleration of the blood flow, this diminution is thought to be a reversal of function brought about by pilocarpine and is more fully discussed in another paper (4). Since pilocarpine apparently interferes with the action of histamine on the blood flow and hence on the secretion, no conclusions regarding the usual effect of histamine can be drawn from these experiments.

Augmented histamine secretion. In an early paper, Dale and Laidlaw (1) described a profuse flow of saliva in the dog and cat, when histamine

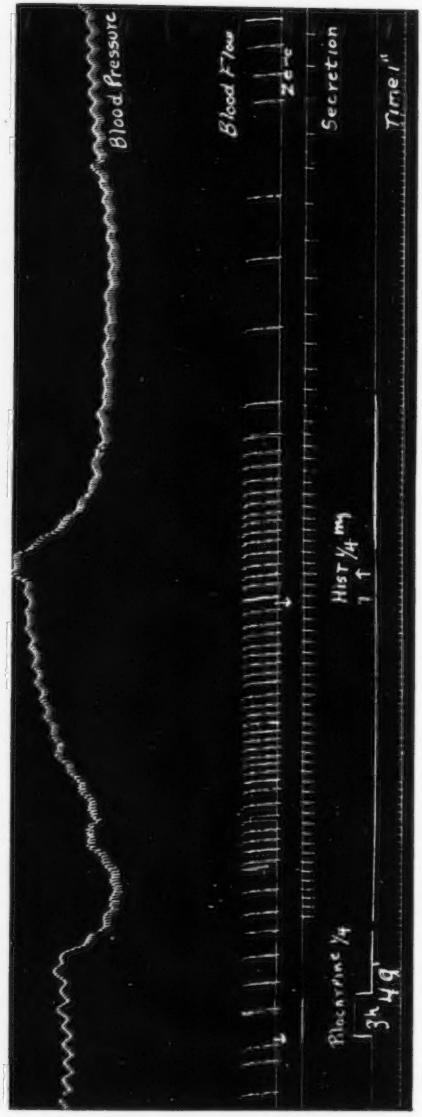

Fig. 1. Cat, chloralose, showing the reversal effect of histamine following pilocarpine

was injected following stimulation of the chorda tympani. On consideration of these experiments, it seemed that the secretion was an augmented effect due to the previous stimulation of the secretory nerves. To investigate this part of the problem a large number of experiments, of which a typical one is cited below, were carried out.

Experiment 3. Cat. Chloralose.

	SECRETION PER MINUTE	BLOOD PRESSURE
Histamine, $\frac{1}{4}$ mgm.	0	120 50–120
2 hours 14 minutes:		
Chorda tympani, coil 11, stimulated for 30 seconds	18	124
2 hours 15 minutes:		
Histamine, $\frac{1}{4}$ mgm.	9	124
1 minute after beginning chorda stimulation		50–120
2 hours 40 minutes:		
Chorda tympani, coil 11, stimulated 60 seconds	33	125
2 hours 42 minutes:		
Histamine $\frac{1}{4}$ mgm.	9	125 48–125

In this experiment a good secretion from histamine was obtained after chorda stimulation, although histamine alone had no effect. This augmented secretion appears to be proportional to the chorda secretion up to a certain point, beyond which it is independent, e.g., in experiment 3, the augmented secretion in two cases was 9 drops after a chorda secretion of 18 drops and another of 33 drops.

In some of the experiments on cats an augmented secretion was not obtained after chorda stimulation. This may be due either to the presence of chloralose, as it was observed that the augmented effect was more pronounced in the decerebrate preparations, or to a poisoning by histamine of the mechanism in the gland responsible for the augmented effect. Much better results were obtained with dogs, as the augmented secretion never failed to appear, although as in cats it may vanish after large amounts of histamine are injected, due probably to the paralyzing effect of this drug on the gland.

This augmented secretion with histamine after nerve stimulation seems to be peculiar to the chorda tympani, because a number of similar experiments were carried out with the sympathetic nerve and only negative results were obtained, except in one case after atropine, where histamine following stimulation of the sympathetic produced a very slight flow of saliva.

On consideration of these facts it is noted that histamine injected before stimulation of the chorda does not influence the secretory effect produced by the stimulation. On the other hand, a previous stimulation of this nerve greatly increases the effect of histamine on salivation.

The following experiments were perofrmed to investigate the mechanism of the action of histamine on the salivary gland. Babkin and McLarren (5), who investigated the augmented secretion from the sympathetic after chorda stimulation, advance the view that it has two phases. These are: a mechanical phase, due to the action of motor fibres in the sympathetic nerve supplying the contractile elements of the gland, and a secretory phase, which is due to an increased secretory response of the gland after stimulation of the parasympathetic nerve. There is reason to believe that there may be two such phases on the augmented secretory effect, which histamine produces after previous stimulation of the chorda tympani.

In the first place, the action of histamine on the motor mechanism is to be considered. Experiments were done on cats and dogs in which saliva was blown back into the gland and then histamine was injected into the blood. The resultant effect was a forcing out of the fluid, probably caused by the action of histamine on the contractile elements of the gland. See experiment 4.

Experiment 4. Dog. 7 kilos.

	SECRETION PER MINUTE	DIVISIONS	BLOOD PRESSURE
	drops		
1 minute before histamine	0	Not recorded	146
1 minute after $\frac{1}{4}$ mgm. histamine	10	Not recorded	44–176
2 minutes after $\frac{1}{4}$ mgm. histamine	2	Not recorded	176
3 minutes after $\frac{1}{4}$ mgm. histamine	1	Not recorded	176
Saliva blown in		70 divisions	
1 minute before histamine	—	—	120
1 minute after $\frac{1}{4}$ mgm. histamine	21	45	40-80
2 minutes after $\frac{1}{4}$ mgm. histamine	5	4	80
3 minutes after $\frac{1}{4}$ mgm. histamine	2	4	98

In this experiment a glass tube was inserted between the cannula and the drop recorder, so that the movement of saliva could be measured on the scale as well as by the drop recorder. The divisions noted above refer to the movement of saliva on the scale per minute, while the drops are those marked by the recorder during the same time. If the amount of spontaneous secretion with histamine is subtracted from the amount of saliva obtained with histamine, after fluid is blown into the gland, it will be seen that there is an excess of 15 drops.

Another form of experiment furnishes evidence that histamine stimulates a certain pressor mechanism in the gland. In a dog the salivary cannula was connected with graduated tubing and a mercury manometer. By raising the pressure in the manometer, the saliva could be forced back into the gland. When the backward movements of the fluid stopped histamine was injected intravenously and a pressing out of the content of the gland was noted. The greatest amount of saliva was pressed out in the first 15 seconds after histamine (+), then gradually diminished after which the saliva passed back into the gland (−). Actual figures of this experiment are as follows:

Experiment 5. Dog.

	DIVISIONS
Massage	5
Pressed back into gland	78
Histamine 1 mgm	Saliva in divisions every 15 seconds: +9, +6, +1, 0, −1, −1, −3, −2, −2, −2, −2, −1, −1, −1.

The pushing out of saliva was obtained in the cat but not to such a marked extent as in the dog experiments similar to the above, the reason apparently being that the motor mechanism is more easily paralyzed by histamine in the cat.

The effect of atropine on the pressing out of saliva by histamine. Two types of experiments were performed, some in which the chorda stimulation was followed by atropine and histamine and others where, after paralysis of the parasympathetic by atropine, saliva was blown back and histamine injected.

It seems that atropine practically abolishes the effect of histamine as in the majority of cases negative results were obtained. In two instances on a cat and dog, however, with the pushing back of saliva a slight reaction with histamine was noted.

True augmented secretion with histamine. The data show that the whole phenomenon of the augmented secretion with histamine cannot be explained by mechanical effects only. On account of this the second phase, namely, a true augmented secretion must also be considered. Such an effect was demonstrated in cats and dogs, where the histamine secretion after the chorda was greater than the actual chorda secretion itself, for instance:

Experiment 6. Cat. 4.1 kilos. Chloralose.

	SECRETION PER MINUTE	BLOOD PRESSURE
11 hours 26 minutes:		
1 minute before histamine	0	120
1 minute after ¼ mgm. histamine	1	88–105
11 hours 30 minutes:		
Chorda, coil 14 for 30 seconds	27	
11 hours 31 minutes:		
1 minute before histamine	0	134
1 minute after ¼ mgm. histamine	22	90–112
2 minutes after ¼ mgm. histamine	6	112
3 minutes after ¼ mgm. histamine	2	114
4 minutes after ¼ mgm. histamine	1	

Further evidence for the secretory action of histamine was gained from the following experiment on a dog. The last injection of histamine gave 7 divisions of saliva, but by subsequent massage of the gland 19 divisions were obtained. After 13 minutes, when the possible effect of the previous injection of histamine had worn off, 53 divisions were blown back into the gland, an injection of histamine then gave 29 divisions and a subsequent massage 20. Now the gland was massaged till no more saliva could be pushed out. Histamine was then injected and gave only 1 division. During massage following the histamine, 13 divisions were pressed out. The figures are tabulated in experiment 7.

Experiment 7. Dog.

	SECRETION DIVISIONS PER MINUTE
3 hours 1 minute:	
Histamine	7
Massage	19
Saliva blown in	53
3 hours 14 minutes:	
Histamine, 1 mgm	29
Massage	20
Massage	1
3 hours 26 minutes:	
Histamine	1
Massage	13

The probable explanation of this experiment is that the amount of saliva secreted under the third injection of histamine filled only the ampulla and ducts of the gland, but was not enough to appear in the main duct.

Gradual diminution of the augmented histamine secretion. The augmented effect with histamine following chorda stimulation may last for 10 to 12 minutes, although its effect gradually diminishes during that time, as occurred in the following experiment, which could be repeated several times on the same animal.

Experiment 8. Cat.

	SECRETION PER MINUTE	BLOOD PRESSURE
Before histamine	0	32
1 minute after ¼ mgm. histamine	0	
12 hours 34 minutes:		
Chorda, coil 14, stimulated for 30 seconds	6	
12 hours 35 minutes:		
Before histamine	0	32
1 minute after 1 mgm. histamine	9	46–40
2 minutes after 1 mgm. histamine	3	40
3 minutes after 1 mgm. histamine	1	40
12 hours 39 minutes:		
1 minute before histamine	0	42
1 minute after 1 mgm. histamine	7	54–50
2 minutes after 1 mgm. histamine	1	50
12 hours 40 minutes:		
1 minute before histamine	0	42
1 minute after 1 mgm. histamine	4	54–50
12 hours 44 minutes:		
1 minute before histamine	0	Clot
1 minute after 1 mgm. histamine	2	

The length of time the augmented secretion with histamine lasts corresponds to the time of the raised excitability of the gland following chorda stimulation.

A possible explanation of the "die away" effect with successive doses of histamine following chorda stimulation is, that the stimulation produces a condition of raised excitability which gradually diminishes and wears off in 10 or 12 minutes. During this period of raised excitability, histamine is able to cause a true secretion, but as the excitability diminishes the action of histamine also becomes less. As the figures above show, the effect must be more than a pressing out, because the augmented effect is at first greater than that due to the chorda stimulation.

Discussion. From the results reported above one may see that histamine has a twofold action on the submaxillary gland; it stimulates the contractile elements and activates the nervo-glandular apparatus of the organ. A previous stimulation of the chorda tympani greatly facilitates the secretory action of histamine, so that the results of Dale and Laidlaw

who obtained a good secretion from histamine injected intravenously following chorda stimulation, are probably due to this phenomenon. The absence of the increased secretion in the cat and dog after sympathetic stimulation may be explained in two ways. First, it may be due, in the dog, to the blocking of the ducts by the very viscid sympathetic saliva; or secondly, the sympathetic nerve and histamine may be partly acting on the same structures in the gland, namely, the contractile elements. It is interesting to note several facts in connection with these results. Histamine, as is well known, provokes a gastric secretion and as Koskowski (6) recently established, a secretion from the small and large intestines. The gastric secretion is not paralyzed by atropine, but that obtained from the intestine is abolished by this drug. Therefore one may conclude that different mechanisms are involved in the secretion of the gastric and intestinal juices under the influence of histamine.

I take this opportunity of expressing my best thanks to Doctor Babkin for his supervision and criticism throughout the course of this work.

SUMMARY

1. Histamine causes in 50 per cent of cases a slight spontaneous secretion in cats and dogs under chloralose. The effect is the same under ether and chloroform anesthesia but is increased in the absence of anesthetics.

2. Histamine injected before stimulation of the secretory nerves does not increase their action.

3. Following a previous injection of pilocarpine, histamine usually gives an inhibition in secretion.

4. Histamine after previous stimulation of the chorda gives in most cases a good secretion with dogs and cats. Atropine greatly diminishes and often entirely abolishes this action.

5. The view is advanced that histamine has a double action on the submaxillary gland; a secretory effect, which is greatly increased by previous nerve stimulation; a mechanical effect due to a pushing out of saliva by histamine acting on the contractile elements in the gland.

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THE RATE OF PASSAGE OF THE MAMMALIAN OVUM THROUGH VARIOUS PORTIONS OF THE FALLOPIAN TUBE

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Sobotta has pointed out that the time required for the ova to pass from the ovarian to the uterine end of the tube is fairly constant in various mammals, regardless of the size of the animal, length of the tube or size of the ovum (Sobotta, 1916). This period has been found to be about three days in the mouse, rat, rabbit, guinea pig, sheep and pig, that is, in all the rodents and ungulates that have been studied. Abundant references to this effect are given by Sobotta (1916). In the opossum, the only marsupial on which the period has been determined (Hartman, 1916), the ova remained in the tube less than 24 hours. In carnivores the time is longer. The dog ovum requires 8 to 10 days (Bischoff, 1845; Bonnet, 1897). Unpublished work by Manwell and Wickens in this department gives substantial evidence that in the cat the period is 6 to 7 days (private communication). Study of the abundant unanalyzed data on the ferret provided by Robinson (1918) shows that the earliest that any ovum was found in the uterus after insemination was $139\frac{3}{4}$ hours. The average period, as calculated from his data, between insemination and the recovery of ova from the tube in the pronuclear stage is 42 hours. There is wide variation of this period, the extremes being $31\frac{1}{2}$ hours and $51\frac{3}{4}$ hours, probably because the pronuclear stage is somewhat prolonged, although we have no data on the point in the ferret. In the mouse the pronuclear stage lasts about 10 hours (Sobotta, 1895), in the rabbit, 12 to 14 hours (Assheton, 1894). Taking 42 hours as our figure for the time between copulation and ovulation, and subtracting it from $139\frac{3}{4}$, the shortest number of hours post coitum at which Robinson found ova in the uterus, we have $97\frac{3}{4}$ as the minimum time required for the ova to pass through the tube. The ferret ova thus enter the uterus early on the fifth day after ovulation. Therefore, in the three carnivores studied the ova are said to remain in the tube varying periods between 5 and 10 days. In the four species of bat studied by Van Beneden and Julin the period seemed to be some weeks, but the evidence was inadequate (Van Beneden and Julin, 1880). To Sobotta's observation that the time required in various species for the ova to pass

down the tube is unrelated to the length of the tube or the relative size of the ova and the tube, we may add the statement that this time is not constant for all mammals but varies widely in the different orders; on the other hand, in the few animals on which we have information, this time is fairly constant for all the species in a given order.

The factors which have been thought to be involved in the propulsion of the ovum along the tube are three: the action of the tubal muscle, the movement of the cilia, and a possible erection mechanism. Many arguments for the importance of each have been brought forward, and numerous references may be obtained from Sobotta (1914, 1916), Grosser (1915, 1918, 1919) and Kok (1926).

Sobotta (1914, 1916) is an advocate of the importance of tubal muscle, and there is additional evidence to this view brought forward by Seckinger (1923) and by Wislocki and Guttmacher (1924). Seckinger, working with isolated rings of muscle from the middle third of the tube of the sow, demonstrated that there are two types of tubal contraction to be found at different phases of the oestrous cycle. That present during oestrus and the first week following ovulation is marked by rapid, undulating contractions, 13 to 15 per minute. During the interoestrous period the contractions are slower, 4 to 6 per minute, and of equal amplitude. This has been fully confirmed by Dr. Jessie L. King in this laboratory (private communication). Wislocki and Guttmacher observed the whole reproductive tract of the sow under warm Locke's solution and verified Seckinger's statements. They also noted that the majority of the peristaltic waves in the tube began at the ovarian end and progressed varying distances along the tube toward the uterus, some going only a few centimeters, and others continuing to the tubo-uterine junction. Occasionally antiperistalsis was seen. The peristalsis was much more vigorous during oestrus and in specimens bearing young corpora lutea. More recently, opposite results have been obtained by Kok (1926) who maintains that the tube is less active during oestrus; and by Von Mikulicz-Radecki (1926) who observed the rabbit's tube in the living animal, and saw no true peristalsis but described a to-and-from movement which progressed very slowly toward the uterus. It may be said that observed activity is more convincing than observed inactivity, for there are many technical factors involved in involuntary muscle experiments which tend to produce the latter results.

The prominence of ciliary movement among the forces which transport the ova along the tube has been emphasized by Grosser (1915, 1919). Their abundant presence in the ampulla of all animals is significant, although they have been shown to be scanty in the ampulla of the mouse (Sobotta, 1895). Snyder was able to find active cilia in the isthmic, ampullar and fimbriated portions of the tube of the sow at all periods of the cycle (Snyder, 1923).

The idea that there may be an erection mechanism in the tube is widespread in the literature and it has been viewed favorably by Graf Spee (1915) and by Grosser (1919). The wide spaces often seen in the center of mucosal folds in sections and noted by Henle (1873), have been shown to belong to the lymphatic system and to have ample drainage and, therefore, can not form an erection mechanism (Andersen, 1927). The blood vessels do not seem sufficient in number or size to uphold the theory of an erection mechanism.

The passage of the ovum might well be impeded by the decreasing diameter of the lumen of the tube, as suggested by Grosser and illustrated in his diagram comparing the size of the ovum with the size of the tube in different regions (Grosser, 1915). In the pig there is also transient edema which is present during the first and third weeks of the cycle (Snyder, 1923). It is especially conspicuous in the mucosa and is accompanied by a dilatation of lymphatics which lasts from the beginning of oestrus to the third or fourth day after oestrus (Andersen, 1927). This dilatation of lymphatics was what impressed the advocates of the erection theory. The edema might intrude upon the lumen of the tube and decrease it greatly. So far, this edema has been observed only in the pig. It is an unregarded factor which may be significant, and should be further studied.

Our knowledge of the relative importance of these factors is still vague, as is evidenced by the many contradictory papers that have appeared in the last two decades. We can still say little more than Bischoff did in 1845—"Fur die bewegenden Kräfte des Eies im Eileiter halte ich die Schwingungen der Cilien des Epitheliums der Eileiter und die Contractionen des Eileiters selbst."

The narrowing of the tube towards the uterine end and the presence of edema during the period that the ova are in the tube at least partially explain why the passage is not made with great speed, but we have yet to discover which of these factors, or what combination of factors carries the ova through the tube in a given time, which has been found unrelated to the length of the tube and the relative size of ova and tube. Neither do we know whether any physiological purpose is fulfilled by this fixed period. The first step toward answering the former question is to find out the speed with which the ova travel through the various portions of the tube. Contributions to the answering of this question have been of two types. Two workers have attacked the problem experimentally. In 1880 Pinner injected granules of various kinds, but chiefly India ink, into the abdomen or in the outer part of the tubes of rabbits, and later recovered the granules from the tube, uterus and vagina. He found that the ink could pass into the vagina in $2\frac{1}{2}$ hours. In unpublished experiments by the author the period was found to be as short as $1\frac{1}{2}$ hours in some cases. The experiment was repeated by Lode on two rabbits (Lode, 1894) with the substitution of

ascaris eggs for ink granules. The ascaris eggs were recovered from the middle part of the tube at 10 and 36 hours after they had been deposited in the abdomen. No eggs were found in the ovarian third of the tube nor in the 15 millimeters next the uterus. Sobotta (1916) ridicules this experiment because the cases are too few and because it seemed impossible to him that no progress had been made by the eggs in the 26 hours between the two experiments and, in addition, considers the results invalid because the ascaris ovum is only one-fourth the diameter of the rabbit ovum. At least it is safe to say that small particles, such as ink granules, have been shown to pass through the rabbit's tube in a few hours, larger foreign objects, such as ascaris eggs, have been found in the middle portion of the tube on the second day, while the rabbit ova themselves are known to require about three days for the journey. This suggests that the delay may be caused by mechanical obstruction depending on the relatively large size of the ova.

The second type of evidence regarding the rate of passage of the ova through the various parts of the tube has been accumulated by culling the literature on early embryology. Many scattered observations have been uncovered, all bearing on either the carnivores—dog, cat and ferret,—or on the rodents—rat and rabbit. The ovum of the dog passes through the first half of the tube in a few hours but stays a long while in the lower part (Bischoff, 1845). The ovum of the cat passes rapidly through the upper two-thirds of the tube and is rarely found there (Van der Stricht, 1911). A study of the data on the ferret provided by Robinson (1918) shows that the ova reach the middle third of the tube soon after ovulation and remain there for about 72 hours. They then require about 30 hours to traverse the uterine third. Ova were found in the middle third of the rat's tube on the second day, and in the uterine third on the third day by Huber (1915) and by H. P. Smith (1917), each reporting but a few cases. Regarding the rabbit, Assheton says, "The embryos, I think, pass rather suddenly through the last 4–6 millimeters of the Fallopian tube at some time between the 75th and 80th hours after coitus." It may be safely said that in all the specimens on which data are available the ova pass rapidly through the ovarian part of the tube and linger in the uterine end. In the case of the ferret and the rabbit it is possible to say more specifically that the ova pass through the ovarian third of the tube in a space of time short enough to insure their rare presence there at autopsy; they spend some days in the middle third and pass through the uterine end more rapidly, in some hours.

In our discussion of the factors involved in the transportation of the ovum through the Fallopian tube, we have not considered the fact that the fertilized ovum itself is undergoing important changes during its passage and these may bear some relationship to the time spent by the ovum in

various parts of the tube. Few observations were found as to whether or not the ova increase in diameter as they approach the uterus. The most definite statement is by Assheton (1894), that the rabbit ova do not increase in size before entering the uterus, although in this species they acquire an investment of albuminous substance. The ova in all the mammals studied enter the Fallopian tube surrounded by remnants of the cumulus oöphorus, which disappear after a variable period and before entrance into the uterus. In the marsupials an albuminous layer is deposited on the ovum and around it a shell membrane, greatly increasing the diameter of the mass. The rapid passage of the ovum into the uterus in the marsupial in spite of this may be explained by the fact that in them the uterine end of the tube has a wide lumen, unlike the isthmus of the Eutheria. Huber notes that the rat ova seen in sections of the isthmus in fixed material are irregular in contour, and suggests that this is due to pressure of the tubal walls.

Efforts to correlate the stage of segmentation with the position of the ovum in the tube, as reported in the literature, resulted in the accompanying table (table 1). The information regarding the stage of the ovum on entering the uterus is in general more reliable than the other information which was sometimes based on few cases. The data were extracted by the author from reports of experiments by the various workers more often than from their statements. It will be observed that, except for the marsupials, the ova enter the uterus between the 4-cell and 32-cell stages of segmentation. It may be that the ova require protection from some influence present in the uterus during their earlier stages—a protection which is supplied by albumen and shell membrane in the marsupials and by delay in the tube in others mammals.

The present experiment was undertaken for the purpose of finding out more accurately the rate of speed with which the ovum passes through the various parts of the Fallopian tube of the sow, both as a means of finding out how the time limit of the journey is so accurately regulated, and in the hope that some contribution might be made to our knowledge of other factors involved in the migration of the ovum through the tube.

Technique. The domestic sow was used because in this species the tube is large enough to handle conveniently, fresh material is easy to obtain in the necessary quantities, and previous work by Corner (1921) on the ovarian and uterine cycles of the sow, and Snyder (1923) and Seckinger (1923) on the tubal cycle provide a good background for further study of the reproductive cycle in the species.

Specimens bearing ovaries with corpora lutea 7 mm. or under in diameter and fresh and vascular in appearance, were obtained. This limit was used because previous experience had shown that ova are not usually recovered when the corpora lutea are over 6 mm. and very rarely when they are over 7 mm. in diameter. The fresh ovaries were described, the diameter and

appearance of the corpora lutea, and the number in each ovary were noted. Each tube was then cut free from its mesosalpinx and straightened. It

The relation of the stage of the development of ova to their position in the fallopian tube in various mammals

ANIMAL	AUTHOR	STAGE OF OVA FOUND IN MIDDLE THIRD	STAGE OF OVA IN UTERINE THIRD	STAGE OF OVA ENTERING UTERUS
MARSUPIALS:				
Dasyrus v viverrinus....	Hill (1910)	—	—	Fertilized but unsegmented
Opossum.....	Hartman (1916)	—	—	
RODENTS:				
Rat.....	Huber (1915)	Pronuclear to 2 cell stage	4 cell stage to 8 cell stage	12-18 cells
				12-18 cells
Guinea pig....	Bischoff (1842)	Pronuclear to 2 cell	4-8 cells	12-16 cells
Rabbit.....	Assheton (1894)	2 cell	5-8 cell	Morula over 20 cells
CARNIVORES:				
Cat.....	R. Van der Stricht (1911)	Passes rapidly through	Pronuclear to morula	28-30 cells
Dog.....	Assheton (1894), Bischoff (1845)	Pronuclear	Beginning segmentation	16-32 cells
Ferret.....	Robinson (1918)	Pronuclear to 8 cells	8 cells	Many celled morula—over 32
UNGULATES:				
Pig.....	Assheton (1898),	—	—	2-4 cells
	Corner (1921)	—	—	2-6 cells
Sheep.....	Assheton (1898)	—	—	8 cells
INSECTIVORE:				
Mole.....	Heape (1886)	—	—	End of segmentation

Fig. 1

was then cut into five equal pieces, measuring from the first widening of the tube as it joins the uterus, to the similar abrupt widening as it joins

the infundibulum (fig. 1). The infundibulum was left attached to the adjacent piece of tube. The end of the infundibulum nearest the uterus was taken as one fixed point because of the great variation in length of the infundibulum. The five pieces were numbered, no. 1 being at the ovarian end, and consisting of the infundibulum and one-fifth of the tube.

Most authors refer to thirds of the tube. Including the infundibulum in our calculation our segment I corresponds approximately to the first third, segments II and III to the second third, and segments IV and V to the third third. The present division was made because it was noticed that the uterine third of the tube included the junction of isthmus and ampulla. This was in segment IV in all but two cases. It seemed desirable to study this region and the uterine end of the isthmus separately. Each piece was washed out with a few cubic centimeters of 0.7 per cent saline into a separate syracuse dish. These washings were then examined for ova, and the records included the number of ova recovered from each segment, and whether they were surrounded by cells from the cumulus oöphorus. The washing out was done within four hours after the material was received, but as the washings sometimes had to wait some hours before being examined, the occasional presence of degenerated ova was not considered significant. No fertilized ova were found in our material.

Observations. The data thus obtained included 84 specimens; that is, 168 tubes. Of the 84 specimens, 32 were discarded, 17 because no ova were recovered from either tube, and 15 because the material was not examined within four hours, or because part of the tube was torn at the slaughter house.

Examination of the records of the 17 specimens in which no ova were found revealed the following:

Corpora lutea 6 mm. in diameter or over, and solid	9
Corpora lutea 45 mm. in diameter and very fresh. In three cases mature unruptured follicles were present	5
Corpora lutea not accurately described	3
	17

The possible reasons for not recovering any ova in those cases and for not recovering 36.8 per cent of ova in the other 52 cases are as follows:

1. The ova may have passed out of the tube into the uterus (probable in 9 cases).

2. The follicles may have ruptured shortly before or during the process of slaughtering and the ova are either not in the tube, or are in the infundibulum, from which they are not likely to be recovered (probable in 5 cases).

3. The ova did not enter the tube, by some mishap in transit from the ovary.

4. They were lost in the washing process.

The fact that in a fair number of specimens bearing large corpora lutea, no ova were found, is evidence that the limit of 7 millimeters diameter for the corpus luteum was sufficiently large to include not only the tubes containing ova but also a safety margin of those whose ova had passed on into the uterus. Otherwise our evidence as to the rate of travel of the ovum would not be altogether valid.

The 52 specimens on which our calculations are based were all those in which all five segments of each tube were washed out within four hours of time of killing, and one or more ova were recovered from one or both tubes.

The number of ova recovered from both tubes of each specimen was compared with the number of corpora lutea in both ovaries and the percentage of ova recovered was found in each specimen, on the assumption that one ovum is produced from each follicle. The average percentage recovered in all 52 specimens was found to be 63.2 per cent. Reasons for the loss of 36.8 per cent of the ova are suggested above. The impression that some of the ova may not yet have entered the tube or may have passed into the uterus is further heightened by calculation of the average percentage of ova recovered from all specimens having ova in the first segment, second segment, and so on, on the assumption that if some ova are on the verge of entering or leaving the tube, others may not yet have entered or may have left it. The results are as follows:

	number of specimens	*per cent ova recovered*
Segment I	3	42.0
Segment II	6	82.0
Segment III	34	71.4
Segment IV	41	65.6
Segment V	16	59.7

The number of specimens having ova in a given segment and the total number of ova recovered from a given segment in all 52 specimens are as follows:

Segment	I	II	III	IV	V	Total
Number of specimens having ova in given segment	3	6	34	41	16	52
Total number of ova in given segment in 52 specimens	3	10	100	160	36	309
Per cent ova in given segment	1.0	3.2	32.4	51.8	11.6	100.0
			84.2			

In 13 specimens (25 per cent) the ova were embedded in a mass of granulosa cells which were easily recognized macroscopically. The average distribution, by segments, of these specimens is very nearly the same as the average for the whole series.

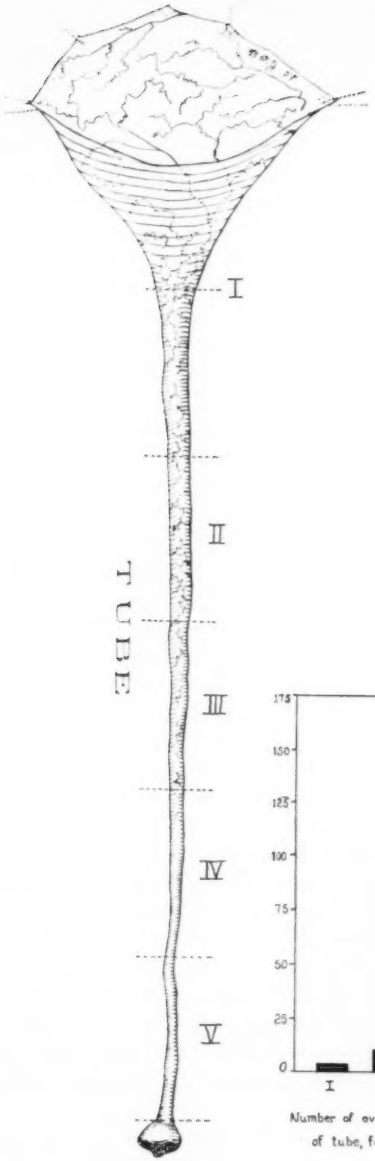

Fig. 2

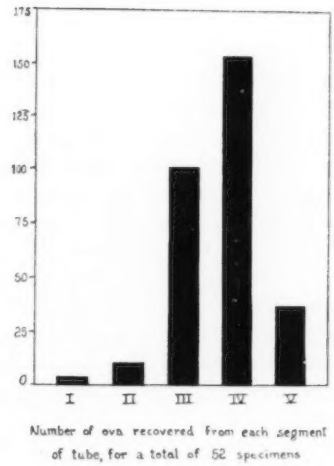

Fig. 3

Fig. 2. Fallopian tube of the sow, divided into five equal segments to show the portion of the tube included in each. ($\times \frac{1}{2}$.)

The finding that 84.2 per cent of all the ova recovered were found in the III and IV segments is evidence that the ova remain longer in this portion of the tube. If the ova progressed through the tube at an even pace, we would find an approximately equal average number in each segment, in such a series. On the other hand, if the ova passed through one portion of the tube rapidly and another more slowly, we would find very few ova in the former region and many in the latter. In a long series the percentage of ova recovered from one portion of the tube would, therefore, represent the proportion of the total time of passage through the tube that the ova spend in that particular portion of the tube. The ova require about three days to pass through the pig's tube (Assheton, 1898; Corner, 1921). Assuming, for purposes of calculation, that this time is actually exactly 72 hours and that the average percentage of ova recovered from each segment of our series of 52 is correct, we can estimate the time required by the ova to pass through each segment with the following results: I, 32 minutes; II, 2 hours 18 minutes; III, 23 hours 20 minutes; IV, 37 hours 18 minutes; V, 8 hours 21 minutes. This estimate cannot, of course, be believed to the minute, but it serves to emphasize the conclusion that the ova pass quickly through the ovarian end of the tube, linger in the middle and pass fairly rapidly through the isthmus (fig. 2).

Discussion. The factors involved in propelling the ova down the Fallopian tube and the time that the ova spend in traversing different portions of the tube have already been discussed. It remains to summarize our knowledge of the different portions of the tube in respect to structure of the tube, condition of ovum while passing through them, and the time-relationships. The general statements made apply only to the Eutheria studied up to date, as enumerated at the beginning of the paper.

The problems connected with the entrance of the ova into the tube are not within the scope of this paper. This entrance takes place in a very short space of time, and some minutes after ovulation the ova are in the ovarian third of the tube. In all mammals in which the point has been studied, with the exception of the dog (Van der Stricht, 1908), ovulation occurs during the stage of the first polar body and surrounded by cells of the cumulus oöphorus. The ovarian third of the tube has active muscle (Seckinger, 1923; Wislocki and Guttmacher, 1924), active cilia (Snyder, 1923) and a wide lumen. The ova are usually fertilized in this region, and spend but a short while, probably some minutes, in passing through to the middle third of the tube.

Discussion of the middle portion of the tube of the sow should concern itself with the uterine half of the ampulla, as the junction of the ampulla and the isthmus falls a little to the uterine side of the junction between middle and uterine thirds of the tube in this species. The uterine half of the ampulla has a gradually decreasing lumen toward the uterus, active

muscle and active cilia. The ova of the sow remain in this region 2½ days, and in all the Eutheria studied the ova spend there the major part of their time in the tube. All available evidence seems to show that they are almost stationary during this time. In all species reported in the literature nearly all of the normal fertilized ova recovered from the middle of the tube were in the stage of two pronuclei or with 2 to 8 blastomeres. The ova studied in the present research were unfertilized. All the ova lose the remnants of cells from the cumulus oöphorus while in this region of the tube.

The isthmus is a relatively short, narrow portion of the tube with a very thick muscle wall. The activity of the muscle has been inadequately studied. There are no cilia on its epithelium in the mouse (Sobotta, 1895). In the pig there are active cilia (Snyder, 1923). The lumen is very narrow. The ova require a shorter time to pass through it than that which they spend in the ampulla. This time is 10 hours in the pig and 30 hours in the ferret. In the lower third of the tube the ova reach the morula stage in all the rodents and carnivores studied and the 4–8 cell stage in ungulates, none achieving the blastula stage before entering the uterus.

These relationships are so constant in all the species of Eutheria studied that they invite hypothesis as to their physiological significance. The only fertilized mammalian ova known to enter the uterus unsegmented are those of the two marsupials studied, Dasyurus viverrinus (Hill, 1910) and Didelphys virginiana (Hartman, 1916), and in these the ova are surrounded in the lower part of the tube by a thick layer of albumen and shell membrane. All Eutherian ova remain in the tube until segmentation is well begun, the time necessary for this varying in different species from 3 days in the pig, to 10 in the dog, with an indefinite number in the bat. It seems a likely theory that the ova are in this way protected during their earliest stages from the more rigorous environment of the uterus.

The question of what causes the delay in the uterine end of the ampulla for so long and so definite a period also tempts hypothesis. The delay might be produced by the cessation or inefficiency of the active forces causing movement of the ova, i.e., muscle and cilia. These forces are known to be continuously active throughout the cycle (Seckinger, 1923; Snyder, 1923). On the other hand, the activity might be of a type unsuited for transporting ova, and of this we know nothing. Moreover, small particles, such as ink granules, pass through very rapidly (Pinner, 1880). The alternative is that there is a temporary obstruction preventing the forward movement of the ova, which might perhaps be produced either by tonic contraction of the isthmic muscle or by edema of the isthmic mucosa. Neither of these factors is understood and both must be studied further.

The helpful advice and criticism of Dr. George W. Corner are gratefully acknowledged.

SUMMARY

1. A series of 168 Fallopian tubes (84 specimens) has been studied. They were obtained from animals whose ovaries bore young corpora lutea under 7 mm. Each tube was divided into five segments, each segment washed out separately with normal saline, and the washings searched for ova. Ova were recovered from 104 tubes (52 specimens). It was found that, counting from the ovarian end, the ova were distributed in the five segments as follows: I, 1.0 per cent; II, 3.2 per cent; III, 32.4 per cent; IV, 51.8 per cent; V, 11.6 per cent; total 100.0 per cent. It was considered that these figures represent the average proportion of the total time in the tube spent by the ovum in traversing a given portion of tube. The progress of the ovum is uneven, and two and one-half of the three days required for the sow ovum to pass from ovary to uterus is spent in the uterine half of the ampulla.

2. The forces which transport the ova through the tube, so far as we know them, are two: tubal muscle and the cilia of the tubal epithelium. Both are known to be active in the ovarian and middle portions of the tube but their activity in the uterine third has been little studied.

3. The time required for the passage of the ovum through the tube was shown to be without relation to length of tube or relative size of ovum and tube, as Sobotta has pointed out, but this time is quite constant for all species of a given order, so far as they have been studied. The time required in the rodents (4 species) is three days; in ungulates (2 species) three days; in carnivores (3 species) 5 to 10 days; in marsupials (2 species), under 24 hours; and in the 4 species of bats studied by Van Benenden, possibly several weeks. In all the Eutheria studied the ova pass rapidly through the outer portion of the tube and slowly through the uterine portion. In the three species on which we have more detailed information, namely, the rabbit, the ferret and the pig, the ova delay longest at the uterine end of the ampulla and the adjacent isthmus, passing more rapidly through the uterine end of the tube.

4. The stage of development of the ova found in various portions of the tube is fairly constant in all the species of Eutheria studied. The ova enter the tube after the first polar body is given off, with the single known exception of the dog, and are fertilized in the ovarian end of the tube. Nearly all the ova found in the uterine half of the tube are in the stages from 2 pronuclei to 2 to 8 blastomeres. The ova enter the isthmus in the stage of 2 to 8 blastomeres in various species and enter the uterus in the stage of 4 blastomeres to many-celled morulae, but never as blastulae.

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Article by Hannah Stillman Bradfield. By an oversight (p. 570) direct reference was not given to Brody's contribution to the subject. This reference is: Brody and Elting, Research Bulletin 89, Agricultural Experiment Station, University of Missouri, 1926.

Am. J. Physiol.

THE DETERMINATION OF THE SURFACE AREA OF WOMEN AND ITS USE IN EXPRESSING BASAL METABOLIC RATE

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The surface area of animals has been studied extensively during the last half-century because area is the best unit of reference so far discovered for comparing the metabolism of different individuals. The area of a few individuals has been measured directly, and on the basis of these measurements many formulae have been devised. Very few women have been measured directly, and therefore it has not been known to what extent the various formulae were accurate for them. It has been observed in this and other laboratories that the basal metabolism of young women was distinctly below the predicted standards and that the average deviation from the Aub-Du Bois standards was greater than that from the Harris-Benedict or the Dreyer standards. It seemed possible that this difference might be accounted for by the fact that the accepted formula for calculating area might not apply to women as well as to men. Recently Brody and his associates have devised an instrument called the surface integrator for measuring directly the area of dairy cows. With this integrator it is possible to measure directly the area of a large number of individuals, since the time needed is much less than for any other direct method.

EXPERIMENTAL METHODS. Forty-seven young women taken at random from University classes were measured and their surface area calculated. The subjects varied in height from 59 to 70 inches; in weight from 89 to 216 pounds; in age from 16 to 38 years; in variation from normal weight from −26 per cent to +78 per cent; however fully two-thirds of the subjects studied were between 62 and 66 inches in height; between 100 and 140 pounds in weight; between −10 per cent and +10 per cent variation from normal weight; and it seems reasonable to regard this as a representative sample of University women. Each of the methods described below was applied to these subjects.

1. *Direct measurement by surface integrator* (Elting, 1925). The integrator consists of a metal cylinder attached to a revolution counter. From the area of the cylinder and the number of revolutions, the surface area can be calculated. A brass cylinder which had an outside diameter of

4.00 cm. and was 4.00 cm. wide was used in this study. The integrator was used on all parts of the body except the head, the area of which was calculated according to Du Bois linear formula, and the fingers and toes which were wound with a centimeter tape line. A great deal of preliminary work was done to check the accuracy of the method. The left and right sides were measured separately, both sides were measured twice on the same day, and the same subjects were measured several times at intervals of a few days. A mold was made on one subject only (subject 23; area by mold, 1.56; by integrator, 1.57). Determinations were made with the subjects standing and lying, and with vertical and horizontal movements of the integrator. None of these variations affected the total result. The preliminary studies were made with the subject dressed in a one-piece bathing suit. After comparing the measurements of several individuals so dressed with those obtained with the subject nude, it was seen that the bathing suit constricted the trunk, and these data were not used. In the final study, all measurements were taken with the subject nude. The left and right sides were measured separately, and if they did not check, the measurements were repeated.

2. *Area by the Du Bois linear formula* (Du Bois and Du Bois, 1915). The nineteen measurements prescribed were taken and the area was calculated from the length and average circumference of the various parts of the body.

3. *Area by the Wörner linear formula* (Wörner, 1923). This formula is based upon slightly different measurements from the Du Bois formula. It is necessary to take seventeen measurements for this formula, and the calculation is somewhat simpler since no constants are used. Measurements are taken from the back with the subject standing, arms outstretched. The area for each part, except the head, is obtained from the formula for the area of a cone. When C is the maximum circumference, and C' the minimum circumference, and S the length,

$$\text{Area} = (C - C')\frac{S}{2}$$

The area of the head and neck equals $4D^2$, when D is the average of 3 diameters.

4. *Area by the Du Bois height-weight formula* (Roth, 1922). The area according to the formula

$$\text{Area} = \text{Weight}^{0.425} \times \text{Height}^{0.725} \times 71.84$$

was obtained from a chart in which weight is plotted by half kilograms and the height by centimeters.

Comparison of methods. The area by the first three methods was calculated to the fourth decimal place, but since it is not necessary for use in

metabolism data to use more than two decimal figures, the results are recorded in all cases to two decimal figures. The results obtained by the second, third and fourth methods were compared with the area by integrator and the percentage deviation calculated. The percentage deviation of the area by each of the first three methods from the area by the Du Bois height-weight formula was also calculated.

METHOD OF DETERMINING BASAL METABOLISM. Sixteen subjects whose area had been measured, were selected for a metabolism study. This group was planned to include some whose area was similar by all methods, and some whose area, as measured by the integrator, differed markedly from the area calculated by the Du Bois formula. The group varied in age from nineteen to thirty-three, the average age being twenty-six.

The usual precautions were taken to secure basal metabolism (Benedict, 1925; Roth, 1922). The Benedict New Portable Respiration Apparatus

TABLE 1

Comparison of area by all methods

FORMULA	NUMBER OF CASES	PER CENT DEVIATIONS FROM INTEGRATOR						
		Range	With regard to sign			Without regard to sign		
			Mean	Standard deviation	Probable error	Mean	Standard deviation	Probable error
Wörner linear	47	−6.8 to +7.3	−0.2	3.2	0.32	2.71	1.75	0.17
Du Bois linear	47	−1.2 to +13.0	+5.7	3.2	0.32	5.60	2.93	0.28
Du Bois height-weight	47	−4.9 to +9.7	+1.8	2.6	0.25	2.63	1.86	0.18

was used, and calculations were made according to Carpenter's (1924) tables. From four to six determinations on different days were made, and results within 5 per cent of the lowest values were averaged.

DISCUSSION OF RESULTS. The areas measured by the integrator averaged 1.8 per cent less than the areas calculated by the Du Bois height-weight formula, and averaged approximately the same as those obtained by the Wörner linear formula. The areas obtained by the Du Bois linear formula averaged 5.7 per cent above the areas measured by the integrator and 3.8 per cent above the areas calculated by the Du Bois height-weight formula. The results are given in charts 1 and 2, and are summarized in table 1.

A study of the data shows that Wörner's linear formula, which does not contain constants, is comparable with the integrator, and that the Du Bois height-weight and linear formulae give results somewhat too high for

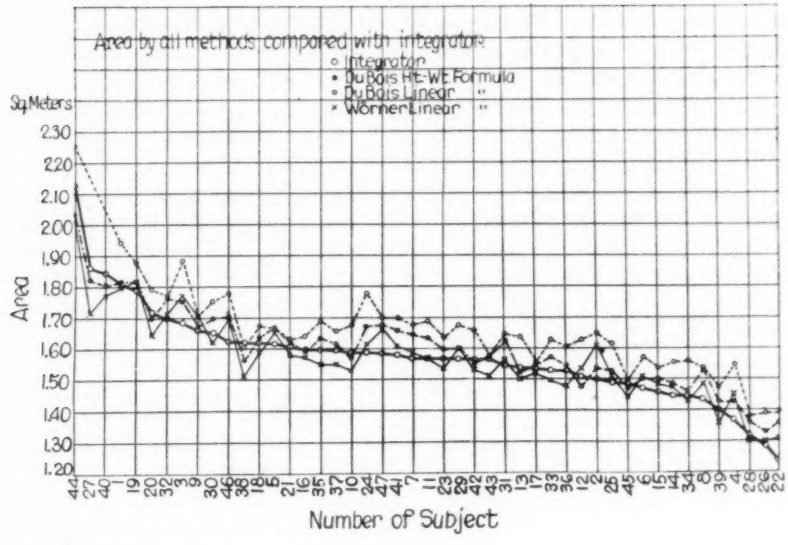

Chart 1

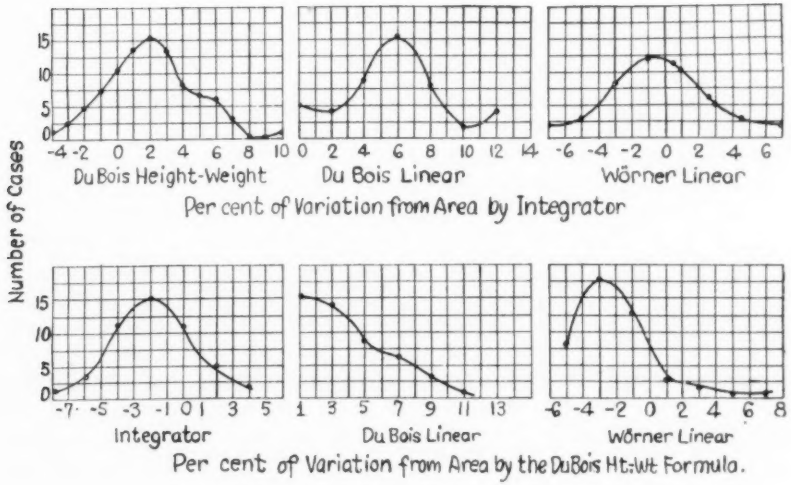

Chart 2

normal young women. A slight modification of the constants is needed to make them applicable to women. Takahira (1925) found a slight modification of the constant necessary in order to make it apply to Japanese men with the same degree of accuracy as to Du Bois' subjects.

It seems probable that greater variation from any formula would be observed in a group of women than in a group of men, due to greater variation in shape. In this study, the range of deviation from all the formulae used was approximately the same (see chart 2), and no new formula based on the data obtained by the integrator is suggested at this time. When weight and surface area by all methods are plotted on logarithmic paper, the areas by the integrator come slightly nearer to a straight line than the area by either of the linear formulae. However, the deviations by all methods are considerable. The line of best fit for the

TABLE 2

Effect of direct measurement of area upon deviation from accepted standards

INVESTIGATORS	NUMBER OF CASES	AGE	CALORIES PER SQUARE METER PER HOUR (AVERAGE)	PER CENT DEVIATIONS FROM STANDARDS					
				Aub-Du Bois		Harris-Benedict		Dreyer	
				A*	B†	A	B	A	B
This study	16	19–33	34.7‡	8.4	−6.1	8.4	−6.1	7.7	−5.1
			33.9§	10.2	−8.3				
MacLeod and Rose	42	20–29	33.8	10.2	−8.5	8.2	−5.4	8.1	−4.8
Blunt and Dye	17	24–44	34.2		−6.5		−4.1		

* Column A, without regard to sign.

† Column B, with regard to sign.

‡ Using area obtained by integrator.

§ Using area obtained by Du Bois height-weight formula.

integrator data gave approximately the same exponent as reported by Elting (1925, 1926). Furthermore when the data were plotted to determine exponents for a height-weight formula, the values obtained were similar to Du Bois'; but again there was so much divergence from straight lines, and the range in size of the subjects was so limited, that it seems that a new formula based on these data would not be a useful contribution.

Further evidence that the Du Bois height-weight formula slightly overestimates the surface of women was found in a comparison of the metabolism data with the standards for prediction which are not reported in full in this paper, but are summarized in table 2. The average deviation from all standards is similar when the area obtained by integrator is used; but when the area by the Du Bois height-weight formula is used, the average deviation is about 2 per cent greater from the Aub-Du Bois standard than from the other standards. This is in accord with the results of Mac-

Leod and Rose (1925) and other investigators (Takahira, 1925; Blunt, 1921), all of whom found greater variation from the Aub-Du Bois standards than from others in the case of young women.

Krogh has suggested a modification of the Du Bois standards which Du Bois states are probably better than the original standards, provided Krogh's specifications are followed (Du Bois, 1927). The data on women students in Columbia University, University of Chicago and University of Missouri are more in accord with Krogh's standards than with those now in use. A study of three hundred tests on sixty-seven University of Missouri women, studies extending over a period of four years, showed that the average number of calories per square meter per hour was 35. The average age of the group was 22 years.

The difference between the Aub-Du Bois standards and Krogh's modification of them probably represents the difference between the procedure in clinics and in experimental laboratories. In the clinics, fewer tests are performed on individuals, and therefore these tests do not represent basal metabolism as nearly as do the tests performed in research laboratories where repeated tests are made and only the lower values averaged. In general, the data from clinics agree with the Aub-Du Bois standards. Enough data have been accumulated now on the basal metabolism of women twenty to thirty years of age to insure that Krogh's modification of the Aub-Du Bois standards fits the data better than the standards now in use.

SUMMARY

A critical study was made of the methods of estimating surface area in order to determine their reliability when applied to women. No study had been made of the surface area of women previously, and it was customary to estimate it from formulae derived from measurements upon men. In this investigation, the accuracy of the surface integrator for human subjects was established by repeated determinations on the same subjects; the area of forty-seven women was determined with this instrument; and the necessary measurements were taken for the calculation of the areas of the same subjects by the Wörner linear, the Du Bois linear and the Du Bois height-weight formulae. The results show that the area obtained by the integrator averages the same as the area obtained by Wörner linear formula; about 6 per cent below the area obtained by the Du Bois linear formula, and nearly 2 per cent below the area obtained by the Du Bois height-weight formula. The range of deviations was about the same by all formulae and was somewhat greater than has been reported for men.

The fact that the difference between the area by the integrator and the area by the Du Bois height-weight formula, though small, is significant is indicated by the metabolism study. The basal metabolism of sixteen of

the forty-seven subjects was repeatedly determined, and the average of the data obtained showed about 2 per cent greater deviation from the Aub-Du Bois standards than from the Harris-Benedict or the Dreyer standards when the Du Bois height-weight formula was used to calculate area. When, however, the area was measured by the integrator, the deviation from all standards was approximately the same.

This study showed that the average basal metabolism was about 6 per cent below that predicted by all standards used, and the same as that predicted by Krogh. This is in accord with previous work which the author has done in collaboration with other students, and also with the work of other investigators, and therefore offers further evidence that all three standards now in use are too high for women of the age range studied.

The results of this investigation indicated that the Krogh modification of the Aub-Du Bois standards should be used for prediction of basal metabolism of women; that if the Du Bois height-weight formula is used to calculate surface area of women, a correction of +2 per cent should be made; and that the maximum error in applying a formula to women even after such a correction is made is ±7 per cent instead of ±5 per cent, the error predicted for men. About 70 per cent of the cases studied would fall within ±3 per cent and about 95 per cent within ±5 per cent.

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THE PROTEIN INTAKE OF MEDICAL STUDENTS

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The first attempt to set dietary standards in terms of food was probably that of Voit, who suggested that 118 grams of protein in a diet containing about 3000 Calories was necessary for a man at moderate work. Chittenden (1), on the other hand, gives little weight to dietary standards in this connection and maintains that the true measure of what the body will most profitably use is to be found in experiments upon protein metabolism, both from the standpoint of the nitrogen balance, and also the daily total nitrogen output over long periods of time. From such studies he concluded that for a man of 70 kilo body weight 60 grams of protein and 2800 Calories per day were necessary.

Since Chittenden's work appeared, much discussion has arisen as to the advisability of feeding a high or low protein diet, and many protein standards have been published. The habitual eating of much more protein than is necessary is very common and is believed to exert a beneficial effect upon health and stamina. Pearl (2) gives 121 grams as the daily protein intake of the people of the United States, while Greenwald (3) gives 113 to 149 grams for the people of several countries. Low protein intakes are indicated in the collection by Sherman (4) of 109 experiments, taken from 25 different investigators, in which 47 individuals served as subjects. The apparent protein requirement ranged between 21 and 65 grams, averaging 44.4 grams per day per 70 kilo body weight. Sherman concluded that an allowance of a gram of protein per kilo of body weight, which is about 50 per cent above the actual requirement, would seem to be adequate.

Several factors may influence the intake of protein. Of these there is no doubt of the effect of the character of the diet. Muscular work does not seem to greatly influence protein metabolism. The effect of temperature has been discussed by Denis and Borgstrom (5), who reported analyses of the total nitrogen in the urine of 233 medical students in New Orleans, over a period of three years, with an average temperature of 13 to 23°C., to be 11.07 grams per 70 kilo body weight. This amount of nitrogen is equivalent to 76.1 grams protein, after adding 10 per cent for that lost in the feces. Borgstrom and Bost (6) studied this question and obtained 10

grams nitrogen per 70 kilo body weight. The above investigators concluded that the protein intake of the South is much smaller than that of the North and that this is due to increased temperature.

The caloric value of the diet will influence very definitely the protein intake of an individual. In this connection Borgstrom, Bost and Hafkesbring (7) stated that the caloric intake of the South does not differ very much from that of the North, but again state that the protein intake is lower. Murlin and Hildebrandt (8) concluded, from their studies of food consumption of large numbers of American soldiers in training camps during the recent war, that the maximum difference between the average food intake for the winter and summer months was not more than 300 to 400 calories.

TABLE 1

Total nitrogen excretion averaged according to years in which the observations were made

YEAR	NUMBER OF SUBJECTS	AVERAGE WEIGHT	AVERAGE URINARY NITROGEN PER 24 HOURS	TEMPERATURE		
				Maximum	Average	Minimum
		kgm.	*grams*	*°F.*	*°F.*	*°F.*
1922	65	71.3	11.00	33.8	27.2	25.1
1923	70	72.8	10.70	35.6	30.2	24.7
1924	68	67.0	11.72	31.4	24.0	16.6
1925	66	66.4	11.11	32.8	25.6	18.4
1926	70	76.1	10.61	34.1	27.2	20.4
1927	61	65.8	11.82	32.8	26.6	20.5
Average..	400	69.9	11.16			

It would seem that a study of a large number of individuals over a period of years should throw light on two important questions in protein metabolism, namely: Does protein intake decrease with increase of temperature, and do certain groups of individuals, for instance medical students, actually consume about 121 grams of protein daily, an amount which has been stated to be the average intake of the nation?

The results given in this paper will attempt to answer these questions. A collection of twenty-four hour urine analyses, obtained for the last six years, from normal male medical students under actual living conditions, has been made by the writer. From about 500 such analyses, 400, which there was no reason to believe were not trustworthy, were selected for study. These analyses for total nitrogen were made in duplicate by the students themselves, after they had mastered the technique of the Kjeldahl method. The diet was the usual one of the student, and the total nitrogen eliminated represents the actual amount of protein eaten during the period of study. All determinations were made during the month of January each

year, and the average temperature was obtained from the local station of the United States Weather Bureau. The ages were from 19 to 30 years. The meals were eaten at restaurants, boarding and fraternity houses, and the University dining hall.

The results are recorded in Table 1 by the years in which they were obtained. The total nitrogen eliminated by the 400 individuals varied from 5.97 to 18.76 grams, with an average of 11.16 grams during a mean temperature range of 24 to 30.2°F. (−1.0 to −4.4°C.). This amount of nitrogen corresponds to 76.7 grams protein per 70 kilo body weight, after adding 10 per cent for that lost in the feces.

As stated above, the New Orleans investigators concluded that the supposedly lowered protein intake of the South was due to increased temperature. My results do not confirm this view, as the average amount of protein eaten, per 70 kilo body weight, by their students and those of this investigation was practically the same.

CONCLUSIONS

A study of 400 twenty-four hour urine analyses of male medical students during the past six years has been made. The results show:

1. An average of 11.16 grams total nitrogen were eliminated, which corresponds to 76.7 grams of protein per 70 kilo body weight, after adding 10 per cent protein lost in the feces.

2. The protein intake of students in the North and South, per 70 kilo body weight, is practically the same.

3. Temperatures between −4.4 to +23°C. have no effect on protein intake.

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THE EFFECTS OF FUNCTIONAL UNION OF THE CENTRAL END OF THE PHRENIC NERVE WITH THE PERIPHERAL END OF THE MOTOR NERVE TO THE STERNOHYOID MUSCLE

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The following experiments were undertaken in the hope of throwing additional light on the difficult problem of the readjustment of central nervous processes in consequence of connecting peripheral nerves with new or abnormal structures. The results are briefly reported because, so far as we are aware, this experiment is new and the ease and simplicity of performing the experiment renders it a suitable teaching demonstration.

PROCEDURE. The operations were done aseptically on five dogs and consisted essentially in suturing the central end of the uppermost root of one phrenic nerve to the peripheral end of the motor nerve to the sternohyoid muscle. One strand of fine silk sutures sufficed to bring the nerve stumps together end to end. The sternohyoid muscle was always selected because of the accessibility both of the muscle and the nerve, a branch of the spinal accessory. To make sure of the phrenic root, electrical stimulation was resorted to before cutting, in every case. The nerves were sectioned in such a way as to leave the sutured stumps long enough to avoid traction on the suture.

RESULTS. The sternohyoid muscles were exposed under paraldehyde anesthesia 3 to 6 months after the operation. Of the five dogs operated, four showed successful functional union, as revealed by the contraction of the muscle on the side of the nerve suture synchronous with every inspiratory act, and proportional in intensity with the depth of respiration. The contractions were, however, not visible nor palpable through the intact skin. They were not strong enough to cause any movement in the subcutaneous tissues, and became distinct only after exposing the muscle and separating it from the muscle of the opposite side. The protocols of the four successful experiments are as follows:

Dog 1. October 4, 1926, suture of central end of cut right phrenic root to peripheral end of motor nerve to right sternohyoid muscle. Explored April 4, 1927, 182 days later. Functional union was established as shown by muscle contraction synchronously with inspiration. The place of suture consisted of a confused network adher-

ing to the surface of the right sternomastoid muscle. Stimulation central to this network caused contraction in the right sternohyoid, and very feeble localized contraction in that part of the right sternomastoid over which the network lay. An effort to dissect the network away from its place of attachment stopped the rhythmical contractions of the sternohyoid. Careful examination showed that large numbers of nerve fibers had penetrated the sternomastoid from the sutured point. One of these nerve strands had established functional union with the sternohyoid muscle.

Dog. 3. January 20, 1927, suture of central end of cut left phrenic root to peripheral end of cut nerve to left sternohyoid muscle. Explored May 18, 1927, 118 days later. Rhythmical contractions in the left sternohyoid were present. The union was almost perfect, and by the naked eye could hardly be distinguished from a normal nerve region. A rubber protected hemostat was applied to produce nerve blocking by compression. Rhythmical contractions disappeared but reappeared after release of the compression. Cutting the nerve abolished the rhythmicity permanently. Stimulation of the peripheral end gave contraction; stimulation of the central stump gave no results.

Dog. 4. February 18, 1927, suture of central end of the cut right phrenic root to peripheral end of cut nerve to the right sternohyoid muscle. Explored June 23, 1927, 125 days later. Rhythmicity in the muscle of the operated side was in evidence. The nerve union was a bulbous enlargement surrounded by abundant connective tissue. Compression of the nerve abolished the rhythmicity. Stimulation central to the crushed portion was negative; but the muscle responded when the distal nerve portion was stimulated.

Dog 5. Nerve suture March 5, 1927. Explored June 17, 1927, 104 days later. The findings were essentially the same as for dog 4.

The results were uniform in demonstrating the rhythmical contractions of the sternohyoid muscle synchonous with inspiration.

COMMENTS. Experiments of cross-anastomosing motor nerves exercising one type of function with those exercising another type indicate that functional restoration occurs, but whether normal coördinated movements are also restored is an open question. The division of opinion on this point seems in many cases to hinge on the differences in the type of experiments performed. It seems reasonable, as Kennedy (1901) is inclined to believe, that for movement under voluntary control some process of reëducation can bring about adjustments tending to eliminate superfluous movements and promote coördination. Successful results have been claimed by surgeons in splitting or crossing nerves to restore function in paralyzed muscles. Still, the literature reports in many instances of successful anastomosis of the spinal accessory with the facial nerve for correction of facial palsy, a tendency to induce facial contortions associated with shoulder movements. To what extent reëducation may eliminate this association is an open question. For involuntary movements the opinions on the question of restoration of coördinated movements are even more conflicting. Alleged good results have been criticised, because the possibility of the development of vicarious function through accessory nerve supply in the muscle involved has not been eliminated.

The experiment reported here involves an essentially involuntary function and has the advantage over the earlier experiments in that the criterion for judging restoration of nerve function is unmistakable. There is no possibility of attributing the rhythmical contractions to development of vicarious function through adjacent nerves. For the time covered by these experiments there is nothing to indicate an attempt on the part of the respiratory center to modify or divide its discharges so as to eliminate the superfluous contractions of the sternohyoid. The longest period of waiting from the time of operation to the time of examination is six months. We are not in a position to state whether a process of reëducation will eventually suppress those useless contractions.

Cannon and his co-workers (1914) reported that by suturing a root of the phrenic nerve to the cervical sympathetic (in cats), they obtained symptoms of hyperthyroidism which were interpreted as the result of continuous bombardment of the thyroid by respiratory impulses going through the sympathetic. Marine, Rogoff and Stewart (1917) repeated these experiments and although they could show functional regeneration of the phrenic-sympathetic union by the presence of a tonic dilator effect on the pupil of the operated side, and by electrical stimulation, they found no pupillary movements synchronous with respiration and no evidence of increased thyroid activity. These experiments raise the possibility that in diverting the phrenic nerve to glandular mechanisms some kind of functional readjustment may lead to suppression of the respiratory impulses over the new channel. In our experiments the respiratory discharges were strong enough to produce good contractions of the sternohyoid and there is every reason to suppose that if such nerve impulses made secretory connections with glands they would induce increased glandular response.

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A STUDY OF THE DIGESTION OF CELLULOSE IN THE WILD RAT

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The object of this work was to study the possible value of cellulose to the living organism of an omnivorous type. Early experiments on man demonstrated that from 4 per cent to 60 per cent of cellulose disappeared or was absorbed, the degree of absorption depending upon the kind and quantity of the cellulose ingested (Weiske, 1870; Knieriem, 1885; Wicke, 1890; Bárány, 1902). Lohrisch (1906) reported that from 79.1 to 81.8 per cent of the cellulose from kohlrabi, 90.5 per cent of the cellulose from spinach, 99.5 per cent to 100.0 per cent of that from white cabbage, 58.8 per cent to 84.8 per cent of that from bread, 95.4 per cent of the cellulose from carrots, and 45.0 per cent of the cellulose from lentils was digested by man. Lohrisch presents a good review of the literature up to that date. Tigerstedt and Tigerstedt (1919) reported the retention or digestion from 5 per cent to 15 per cent of different kinds of hydrated cellulose in an extensive series of experiments on man.

Just what is the fate of the retained cellulose and its value to the omnivorous animal has been a point in question for some time. Tappeiner (1884, 1888) found that when cotton-wool was soaked in a 1 per cent solution of bouillon and the resulting mixture inoculated with intestinal bacilli, gas (CO_2 and CH_4) was evolved and free fatty acids (acetic, butyric, valerianic) were found in the solution. Nearly all of the cotton-wool product was dissolved. Hoppe-Seyler (1886) using filter paper and pond bacteria obtained CO_2, CH_4, and a dextrin-like substance but no fatty acids. Bunge (1902) suggests that the epithelial cells of the intestine may possess a substance which, by fermentative action, enables these intestinal cells to dissolve some of the cellulose, supporting his contention by referring to the work of Cienkowski who found that the unicellular organism, Vampyrella, dissolved the cellulose wall of the algae, Spirogyra. However, as yet, no such substance or ferment, as suggested by Bunge, has been found. The recent work of Khouvine (1923) presents the first successful method for isolating a microörganism from the intestinal flora of man which when cultured on cellulose caused it to be broken down and dissolved. This organism was strictly anaerobic and was difficult to

culture. It was found to grow normally only where the nitrogenous matter is much reduced as in fecal extracts. When cellulose was subjected to the action of the isolated bacilli, the products were carbon-dioxide, hydrogen, ethyl alcohol, acetic acid and a yellow pigment. This investigator was also able to detect the presence of lactic acid and some products of hydrolysis which were precipitated by alcohol. The bacillus, in a pure state, was found to break down less cellulose than when other types of microörganisms were present along with it. Since this is the situation in the digestive tract, the cellulose of the ingested food may be split up more rapidly and in greater amounts. Pringsheim (1912) succeeded in showing that a bi-hexose and glucose were formed as intermediate products of the action of bacteria on cellulose (filter paper) and according to Bayliss (1924), "there seems to be no doubt that the glucose is, in great part, absorbed from the alimentary canal before the bacteria are able to complete its destruction or convert it into more degraded products, such as hydrogen or marsh gas."

Preparation of the cellulose product. Celotex was used as the source for cellulose. This is the commerical name of insulating lumber which is manufactured from the residual material of sugar-cane (Bagasse); that is to say, it is prepared from the material which remains after the cane has been subjected to the process of removing its sugar content. The residual matter, thus, consists mostly of cellulose. Since the Celotex, as purchased from the market (lumbar yards), is in the form of a board and since the fibrous nature is extremely coarse, it was necessary to put the preparation through a mill in order to convert it into a flour.

Aqueous extracts of the cellulose-flour always gave the Molisch reaction. The iodine test for starch was faintly positive only when the flour was extracted with boiling water. In a concentrated aqueous extract (1 cc. representing 1 gram of the flour) the Benedict test for reducing sugar was faintly positive, 6 cc. of the concentrated product resulting in the formation of a slight red precipitate upon cooling. With respect to the Benedict's test, it is entirely possible that the process of concentrating the product by boiling resulted in the hydrolysis of some of the higher saccharides and, as a consequence, a positive test, as indicated, was secured. The biuret was always negative while the xanthoproteic was positive. This suggests that the product contains no protein material but does have some amino-acids, some of which, as is evident from the xanthoproteic test, possess the benzene nucleus.

The amount of reducing sugar present in various extracts of the Celotex was determined by the method of Hagedorn and Jensen (1918). When the material was extracted with boiling water the average amount was 0.147 per cent per dry substance. Extraction with water at room temperature for the same length of time (2 hours) yielded 0.076 per cent per

dry substance. The increase in the sugar yield in the boiling water extract is probably due to the swelling of the cane fiber resulting in a better penetration of the water. It does not seem likely that any of the higher saccharides were hydrolyzed or broken down to the simple sugars or monosaccharides by this treatment. With respect to bacterial action, such was eliminated in the extracts where heat was used while in the preparations, extracted at room temperature, the time was too brief and the conditions were not favorable, since in the aqueous extracts, at room temperature for 24 hours, the average amount of reducing sugar (0.120 per cent) is practically the same as that for the extracts in which heat was used.

Table 1 gives the average amount of amino-nitrogen and total nitrogen found in various extracts and digests of Celotex-flour. The percentages

TABLE 1

Average amount of amino-nitrogen and total nitrogen in various celotex extracts and digests

KINDS OF EXTRACTS OR DIGESTS	AMINO NITROGEN		TOTAL NITROGEN	
	Gram per cubic centimeter of extract	Per cent	Gram per cubic centimeter of extract	Per cent
Aqueous extracts	0.00001545	0.0261	0.00009215	0.1101
Acid extracts	0.00002390	0.0287	0.00003817	0.0458
Alkaline extracts	0.00002298	0.0275	0.00003850	0.0462
Acid-pepsin digests	0.00002980*	0.0357	0.00002108*	0.0253
Alkaline-pancreatin digests	0.00000953*	0.0115	0.00000726*	0.0090

* The amino-nitrogen and total nitrogen of the digestive products or enzymes, pepsin and pancreatin, have been deducted. The above results are net values coming entirely from celotex.

are calculated on the basis of the dry product. The amino-nitrogen was determined by the Van Slyke (1912, 1913) method, using his micro-apparatus, and the total nitrogen by the Arnold-Gunning modification of the Kjeldahl method. The aqueous preparations were extracted at room temperature for 21 hours while the acid (0.3 per cent HCl) and alkaline (0.17 per cent Na_2CO_3) were incubated at 38.0°C. for 42 to 43 hours. The amino-nitrogen content of the aqueous extracts was 0.026 per cent; for the acid, 0.029 per cent, and 0.028 per cent for the alkaline. The total nitrogen in the aqueous extracts was found to be 0.110 per cent; for the acid, 0.0458 per cent; and for the alkaline preparations, 0.0462 per cent. The total nitrogen content of the Celotex board was 0.323 per cent. The foregoing data indicate that the nitrogenous material may be chiefly in the form of amino-acids.

Digestion experiments. In 18 experiments, using 50 mgm. cellulose-flour per cubic centimeter of saliva, at 38°C. for 39 to 41 hours, there was an average of 0.054 per cent increase in reducing sugar, with only slight variations in the individual experiments. In 8 experiments, using commercial pancreatin, there was no increase in the reducing sugar. Sugar determinations were made on all the pepsin-HCl digests of the cellulose-flour. These digests gave a higher sugar content (0.120 per cent) than the digests with human saliva, probably due to acid hydrolysis.

The effect of pepsin (6 experiments) and pancreatin (6 experiments) on the nitrogenous material in Celotex is summarized in table 1. In the pepsin series, the Celotex-flour was incubated with a 1 per cent pepsin in 0.3 per cent HCl solution for 42 hours at 38°C. There was no increase in either the amino-nitrogen or total nitrogen content of the digests. The average amounts or percentages for amino-nitrogen and total nitrogen are practically the same as those found in the aqueous, acid and alkaline extracts. However, in the pancreatin series (flour incubated with 1 per cent pancreatin in 0.17 per cent Na_2CO_3 solution for 42 hours at 38°C.), a marked decrease in both amino-nitrogen and total nitrogen was found, probably due to bacterial action.

From the foregoing results, it is evident that the cellulose was not attacked by the digestive preparations, namely, saliva, pepsin and pancreatin. The slight increase in the reducing sugar of the saliva experiments was the result of the action of the salivary enzyme on the starch content of the Celotex-flour, this being present in very small quantity. The somewhat greater increase in the reducing sugar in the pepsin experiments was probably due to hydrolysis by an increase in acidity rather than by the pepsin.

Fermentation experiments. The Celotex-flour was mixed with distilled water (from 0.5 to 1.0 gram of the flour per 20 cc. of water). To this mixture was added either the yeast suspension ($\frac{1}{2}$ cake of yeast per 10 cc. of water) or the culture of bacteria (B-aerogenes, B-coli cystitis). In the case of the yeast suspension, 2 cc. were usually added. The resulting product was thoroughly mixed and transferred to fermentation tubes, the arms of which were graduated in cubic centimeters. The volume of air in the bulb was next determined by placing a flat object over the opening in the bulb and transferring the air to the graduated arm, care being taken not to exert any pressure in the tube. The volume of the air was noted. After this, the air was transferred back to the bulb and the opening of the bulb was tightly corked, the cork being sealed and wired in place so as not to permit it to be blown out. The air was again transferred to the arm and the volume recorded. Control tubes, one consisting of the same amount of yeast suspension and water or bacteria and water, and the other, of the same weighed amount of Celotex-flour in the same volume of water as was

used in the Celotex-yeast or Celotex-bacteria preparations (no Celotex present in the first and no yeast or bacteria in the second), were prepared in exactly the same manner. In this particular work, all the tubes were put into the incubator at a temperature range of 38.0° to 40.0°C. for an average of 116 hours. At the end of this time, the gaseous mixture in the bulb was introduced into the arm from which the volume was read. Subtracting from this volume that which was observed when the tube was sealed, gives the amount of gas formed under pressure. This, as a rule, was under quite a good deal of pressure in the yeast preparations and less so in some of the bacteria-mixtures. Then, without putting the gaseous mixture back into the bulb, the seal was broken and the cork slowly removed, allowing the pressure, if any, to escape gradually. With this done, the volume was again observed. The difference between this volume and that one observed before the tube was sealed is the amount of gas formed, which, in this case, is at atmospheric pressure. This method is not absolutely quantitative, since some of the gas is in solution. However, it affords a way of securing a fairly good estimation for comparison. Using the above procedure, it was found that the B-coli cystitis formed no gas when incubated with Celotex-flour, while B-aerogenes did. The latter caused about 1 cc. of gas to be formed, this being the volume at atmospheric pressure. In the case of yeast, it was found that when sterile precautions were taken, that is, using sterile water in making up the preparations and sterilizing the fermentation tubes and corks, etc., there was just as much gas formed in the controls as in the Celotex-yeast preparations. However, when no sterile precautions were taken, there was more gas formed in the Celotex-yeast products than in the controls, this being on an average of 2.2 cc. at atmospheric pressure. It is apparent, from this, that when sterile precautions are taken, some organism is removed which plays an important rôle in the action on cellulose by yeast. More work on this particular question is necessary.

Feeding experiments. The animals used in this work were wild rats. The wild rat was selected as subject for the experiment since its natural diet is of a varied nature. Before put on experiment, the rats were kept for two weeks on a diet, "table scraps," to permit the animals to acclimate themselves to cage life and to enable the selection of normal subjects for the work. The animals were kept in individual cages during the experiment. The chief point in this work was the influence of various quantities of Celotex-flour in the diet. The diets which were used are as follows:

Diets

1. A mixture of ground malted milk bread, cornmeal, cheese in the ratio of 50, 30 and 20.
2. A mixture of diet 1 and Celotex-flour in the ratio of 1:1.
3. A mixture of diet 1 and Celotex-flour in the ratio of 1:2.

4. Celotex-flour moistened with water.
5. Coarse Celotex-flour moistened with water.
6. Dry Celotex board.
7. White pine sawdust.
8. Diet 1 fed in amounts which were present in the quantity of diets 2 and 3 ingested by the rats.

Each ration (2–5) was fed in excess of consumption. One-tenth (0.1) of a gram of diet 1 per day per gram of body weight was found to keep the animals in fairly constant weight. The rats on diet 2 lived, as shown in the chart (fig. 1.), an average of 7.1 days (minimum 4—maximum 11); those on diet 3, 5.4 days (minimum 2—maximum 10); those on diets 4 and 5, 5 days (minimum 4—maximum 7); those on diet 6, 6.3 days (minimum 5—maximum 8); those on diet 7, 7 days (minimum 5—maximum 10); and the animals on diet 8, 6.9 days (minimum 4—maximum 11).

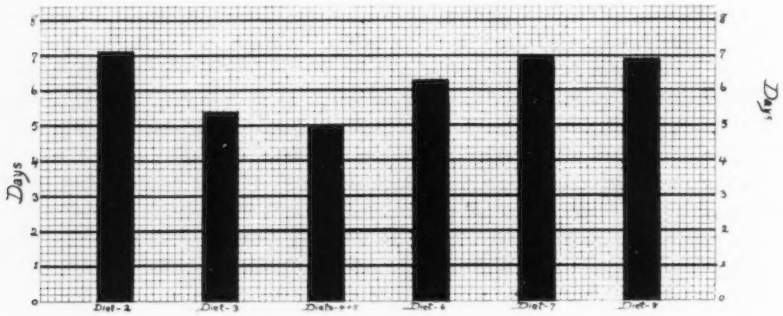

Fig. 1. Showing the average survival of the wild rats on the diets indicated

The animals varied from 60 to 260 grams in weight. Each experimental group included animals which covered, approximately, this range. It was observed that the smaller (younger) rats lost weight more rapidly, due probably to their high rate of metabolism. The animals that were relatively quiet in the cages lost weight more slowly and survived the longest. When the rats were grouped according to weight or age, it was found that for those under 100 grams, the average loss in weight before death was 21 grams and the average length of life, 6.4 days; rats weighing between 100 to 200 grams lost 44 grams before death and lived 7.9 days, while the heaviest animals (224 grams, av.) lost 68 grams and survived 7.0 days. Thus, on the inadequate diets, the animals died when $\frac{1}{3}$ of the original body weight was lost.

Autopsies were made on all of the animals, paying special attention to the condition of the alimentary tract. The small intestine appeared inflamed in eight cases. In two of these, the stomach was also inflamed and

in another, the colon. Four of these animals were on diet 2; two, on diet 3; one, on diet 4 and the other on 5. A small amount of blood was present in the intestinal contents of five of these animals (two on diet 2 and the ones on diets 3 and 4). In seven other cases, blood was found in the intestinal contents and in another, in the content of the stomach, but no other indication of inflammation. Two of these animals were on diet 5; one on diet 4; two, on diet 8 and three were on diet 3, one of the latter having blood in the urine. In one case, the inflamed areas contained much Celotex material. The tracts of two other animals on the same diet contained much of the ration (Celotex) throughout the entire course. There was no evidence of inflammation.

The deaths of the rats on diets 6 and 7 were due to starvation since the animals ingested none of their respective diets at any time during the experiment. In comparing the length of life of the animals on the other diets (2, 3, 4, 5 and 8) with that of the above animals, a significant variation will be found. The rats on diets 3, 4 and 5 lived two days less than those on 2, 6, 7 and 8. The intestinal tracts of all of the animals on diets 3, 4 and 5 were inflamed or of a hemorrhagic nature. It appears that the deaths of these rats may have been the result of enteritis rather than starvation since they survived a shorter period. A similar condition was found in about 40 per cent of the animals on diet 2, while 14 per cent of those on diet 8 showed traces of blood in the intestinal contents.

SUMMARY

1. The cellulose preparation, used in this work, was found to contain small amounts of reducing sugar and nitrogenous material, the latter being chiefly in the form of amino-acids.

2. Cellulose was unattacked, in vitro, by digestive enzymes.

3. B-aerogenes and yeast incubated with cellulose-flour resulted in the formation of gas, the latter, however, only when no sterile precautions were taken. B-coli cystitis formed no gas on the cellulose media.

4. It appears that when sterile precautions are taken, some organism is removed which plays an important rôle in the action on cellulose by yeast.

5. The cellulose of the sugar-cane, in the form of Celotex, is of no nutritive value to the wild rat and when ingested in large amounts, is harmful, causing inflammation of the gastro-intestinal tract.

We wish to express many thanks to Dr. David Klein of the Wilson Laboratories who so kindly supplied us with the pepsin and pancreatin preparations; and also to Dr. E. J. Van Liere for valuable technical assistance rendered during the investigations.

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THE REGULATION OF RESPIRATION

XI. Effects of Changes in Alveolar Oxygen Pressure on Tissue Acidity and Blood Acidity

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This paper is a further effort to elucidate the respiratory response to variations in the oxygen content of the inspired air. It is now obvious that the response to changes in alveolar oxygen cannot be reconciled with views that were held on the stimulating action of the free hydrogen ions of the arterial blood, for the relation is inverse rather than direct. Furthermore, a directional relation between blood acidity and pulmonary ventilation had never been established for it has been shown (Gesell, 1923; 1925) that ventilation may be intensively stimulated in various procedures though the blood swings alkaline or acid, or it may be completely inhibited though the blood swings acid or alkaline. Nor is there a simple relation between alveolar carbon dioxide and pulmonary ventilation which can be used to explain the response to low alveolar oxygen, for the inverse relation between pulmonary ventilation and alveolar carbon dioxide is as easily demonstrated on the administration of air poor in oxygen as is the direct relation under other conditions. Neither is there a simple relation established between alveolar oxygen and pulmonary ventilation, for high alveolar oxygen may be as effective in stimulating ventilation as low alveolar oxygen (Gesell, 1923).

In an effort to arrive at a more comprehensive understanding of respiratory control one of us advanced a theory which involved the acid metabolism of the respiratory center itself, the transport of acid from the center and the resulting acid relations in the center. In applying this theory to the stimulating effect of low alveolar oxygen a change in acid metabolism in favor of increased formation of lactic acid was suggested. The impaired oxidation of hemoglobin in the lungs impairs reduction of the hemoglobin in the tissues. There is a broken coördination of the dual function of hemoglobin. The acid formed is without an adequate vehicle for transport and consequently remains in the tissues. The acid relations in the center are changed.

Apparently tissue acidity and tissue oxidations are intimately related.

Though aware that numerous factors are continually operating in the control of pulmonary ventilation (changes in pH, oxidations, distribution of various ions, etc.)—for the rhythmic discharge of the respiratory neurones may be regarded as an electrochemical phenomenon in which the composition and changing composition of a membrane and the fluids on each side of that membrane determine the nature and frequency of the discharge (Gesell, 1926)—we will deal in this paper primarily with the hydrogen ion.

Since respiratory activity and acidity of the interior of the respiratory neurone appear to show a common direct relation, and since respiratory activity and acidity of the blood may show a common inverse as well as a direct relation, depending upon the conditions supplied, the acidity of the interior of the cell stands out more obviously as a controlling factor of pulmonary ventilation. Nevertheless, assuming that the general postulate concerning the significance of the properties of the fluid on both sides of the respiratory neurone membrane holds, an effort must be made to learn the changes in the immediate cell environment and then to relate intra and extracellular changes with respiratory activity. In these experiments we have made direct measurements on the blood alone. The changes in acidity of the interior of the cell can only be inferred. And as immediate environment of the cell is a product of the neighboring blood and the activity of the cell, inferences relating to its properties are less accurate. Consequently we can only hope to suggest the possibilities relating changes in the immediate cell environment to associated changes in pulmonary ventilation.

The methods of study which were used are similar to those reported in previous papers. Oxygen mixtures in nitrogen were administered with rebreathing tanks by normal ventilation and by artificial ventilation. Changes in ventilation and oxygen consumption were recorded along with changes in acidity of the circulating arterial and venous blood. The changes in acidity were followed with the manganese dioxide electrode—one placed in the carotid artery and the other in the external jugular vein.

In the use of this electrode we call attention again to the fact that it is an oxidation-reduction system; that it is, therefore, sensitive to reducing substances as well as to changes in hydrogen ion concentration. The E.M.F. of such an electrode placed in the blood stream may consequently be a resultant of two chemical influences. Whether or not it will function as an indicator of changes in acidity will be determined by fluctuations in the oxidation-reduction factor. If the acid changes in the blood exert a dominant effect the electrode will indicate changes in acidity. If the other factor becomes predominant the electrode behaves primarily as an oxidation-reduction electrode. It is, therefore, imperative to check the behavior of the electrode in the blood stream for very little is known of the liberation

of reducing metabolites by the tissues into the blood. In our first paper on the method (Gesell and Hertzman, 1926) we reported good directional agreement on the administration of air poor in oxygen between the behavior

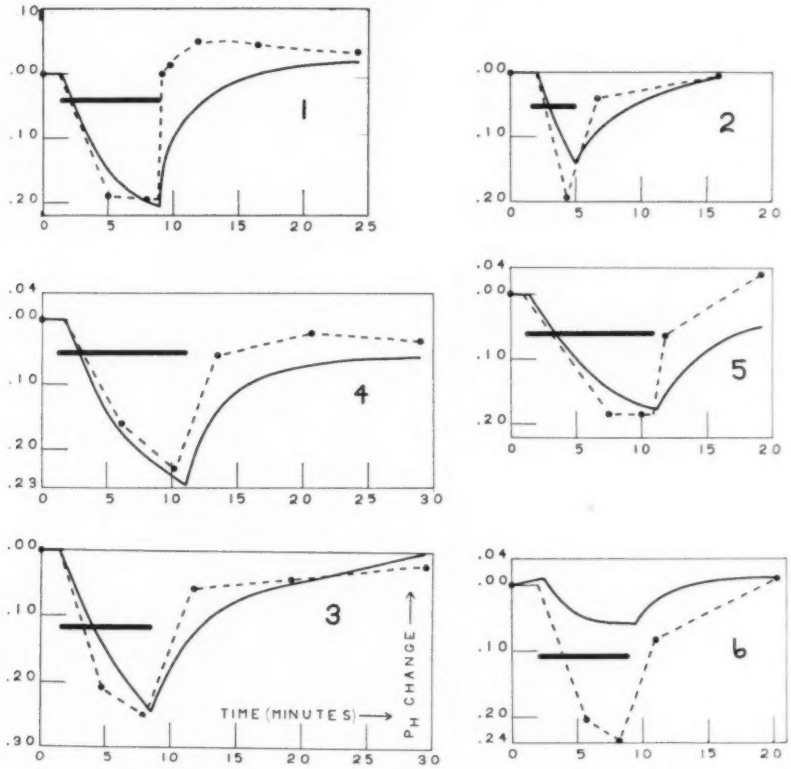


Fig. 1. A comparison of acidity curves of the circulating blood recorded with the manganese dioxide electrode and established by blood samples with the hydrogen electrode showing the effects of administration of 4 per cent mixture of oxygen in nitrogen. Changes in pH are plotted on the ordinates against time in minutes on the abscissas. The duration of the administration of low oxygen is shown by the solid horizontal bar. Graphs 1, 2 and 3 show changes in arterial blood, and 4, 5 and 6 in venous blood. In graphs 1 and 5 oxygen instead of room air is administered after 4 per cent oxygen. Graph 6 shows the sluggish behavior of the manganese dioxide electrode when covered with a thin fibrinous clot.

of the manganese dioxide electrode in the circulating blood and the quinhydrone electrode in corresponding blood samples. Aware of the possibility of the quinhydrone electrode being subject to the same chemical

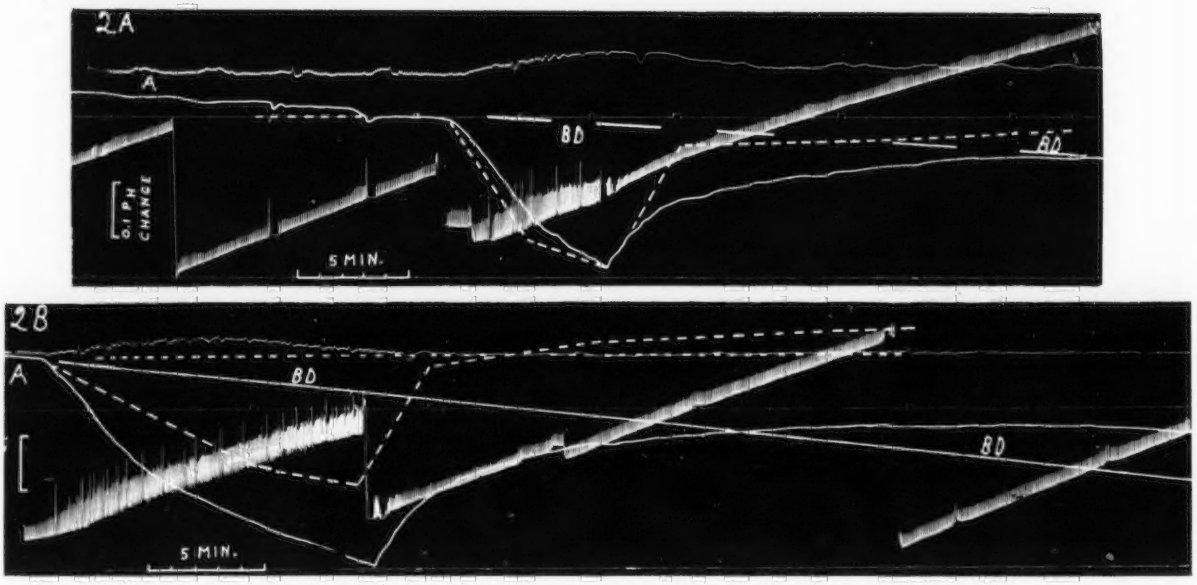


Fig. 2. Original records of changes in acidity of the circulating arterial blood showing associated changes in mean blood pressure and pulmonary ventilation. The basal drifts, *B.D.*, of the manganese dioxide acidity curves are extended. The changes in acidity as established with the hydrogen electrode on blood samples are shown in the broken curve.

influences as the manganese dioxide electrode, for it is also an oxidation-reduction system (Cullen and Bülman, 1925), we suggested the advisability of further checks in various types of animal experiments—particularly those involving severe disturbances in oxidation.

Such checks have been conducted in these experiments on the effects of low alveolar oxygen on blood acidity and are shown in figure 1. The manganese dioxide acidity curve (continuous curve) which is continuously registered on the smoked record is replotted for comparison with the results established with the hydrogen electrode on discontinous samples (broken curve). Examples of original smoked paper records showing changes in blood acidity, mean blood pressure, pulmonary ventilation and time in 5 second intervals are shown in figure 2A and B. The continuous curve marked *A* shows changes in acidity of the circulating arterial blood as recorded with the manganese dioxide electrode. The basal drift, *BD*, of the electrode is obtained by extending the gradient of the manganese dioxide acidity record preceding the administration of a 4 per cent mixture of oxygen in nitrogen. When the correction for drift is made it will be noted that in this particular record there is excellent agreement between the manganese dioxide and hydrogen electrode (broken curve). At the end of the record—about 15 minutes after the readministration of room air—the arterial blood is slightly more alkaline than immediately preceding the administration of low oxygen, as indicated by both electrodes. Figure 2B is a similar record showing good agreement between the manganese dioxide and hydrogen electrodes. In this record it will be noted that both electrodes show final acidity greater than the initial acidity.

One point which is brought out in this and the six graphs of figure 1 is well to keep in mind in the interpretation of manganese dioxide acidity curves; namely, the lag in the response to changes in acidity. This appears both on the administration of low oxygen and on the readministration of room air. It is especially well illustrated in graph 1 of figure 1. In that observation pure oxygen was administered following the administration of 4 per cent oxygen. This accounts for the rapid increase in acidity of the arterial blood which the manganese dioxide electrode failed to follow.

The six observations shown in figure 1 were made on five different animals (4 and 6 on the same dog). In each observation a 4 per cent mixture of oxygen in nitrogen was administered by normal ventilation as in figure 2. In graphs 1, 2 and 3 changes in acidity of the arterial blood are plotted, and in graphs 4, 5 and 6 changes in acidity of the venous blood. The acidity changes are plotted on the ordinates in 0.1 pH from a zero value as base, upward deflection indicating decreasing pH. Time in minutes is plotted on the abscissas. The horizontal bar shows the duration of the administration of low oxygen. In graphs 1 and 5 pure oxygen

is substituted for the readministration of room air. This accounts for the final higher acidity. Graph 6 shows the poor behavior of the manganese dioxide electrode when covered with a thin fibrinous clot. Granting the dependability of the hydrogen electrode under the conditions provided in

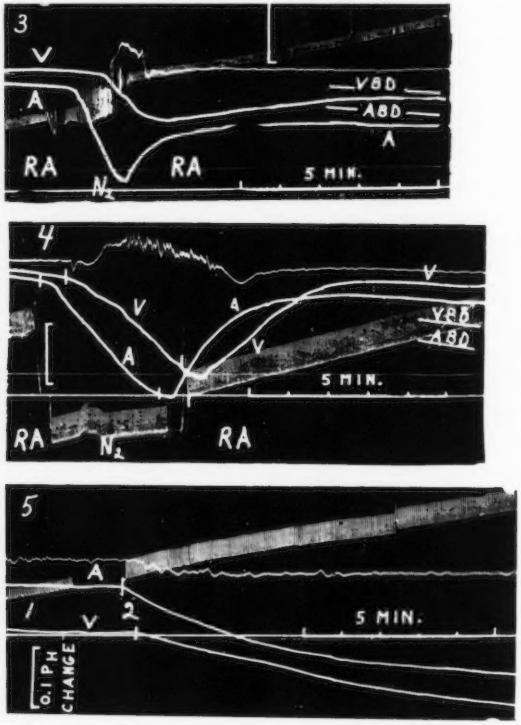


Fig. 3. Simultaneous records of changes in acidity of the circulating arterial and venous blood resulting from temporary administration of nitrogen by normal ventilation. The basal drift of the arterial and venous electrodes is extended.

Fig. 4. Simultaneous records of changes in acidity of arterial and venous blood resulting from temporary administration of nitrogen by normal ventilation. The basal drift of the arterial and venous electrodes is extended.

Fig. 5. Effects of increased pulmonary ventilation on the acidity of the arterial and venous blood.

these observations, the validity of the manganese dioxide electrode is indicated.

Results. Figure 2 is a common response to a temporary administration of a 4 per cent oxygen mixture. The arterial blood turns alkaline, in this

instance about 0.2 pH; pulmonary ventilation and mean blood pressure increase. On the administration of room air the blood turns acid and pulmonary ventilation and mean blood pressure subside. Figure 3 shows a similar response to a temporary administration of nitrogen, and in addition shows a synchronous record of acidity of the venous blood of the same directional change. Though theoretically the probability of the tissues turning acid as the blood turns alkaline, and later the tissues turning alkaline as the blood turns acid is indicated—thereby accounting for the changes in respiratory response—these records by themselves fail to establish such evidence.

In figure 4 the conditions of the experiment are slightly altered. Room air and nitrogen are administered by uniform artificial ventilation during pneumo-thorax. The changing respiratory movements, therefore, have no effect on pulmonary ventilation. On the administration of nitrogen both the arterial and venous bloods turn alkaline, which suggests at once that only part of the increased alkalinity of the blood in figure 3 can be attributed to increased pulmonary ventilation. On readministration of room air the arterial and venous bloods turn acid and to a higher level than the initial value. Inasmuch as pulmonary ventilation remains constant it is evident that the increased acidity of the blood on readministration of room air with normal ventilation cannot be attributed to decreased pulmonary ventilation. Apparently the changes in acidity of the circulating blood with constant ventilation are largely a function of the reduction and oxidation of the hemoglobin.

As a rule the blood turns alkaline more rapidly and more extensively when low oxygen is administered by normal ventilation, but it turns more acid (higher acid level) on the readministration of room air with artificial ventilation. These differences in acid changes of the blood with normal and artificial ventilation are sufficiently pronounced to appear though the individual observations may be made on different animals, as is the case in figures 3 and 4. They are apparently explainable by the differences in pulmonary ventilation. Certainly the increased pulmonary ventilation obtaining with normal ventilation must lead to a greater elimination of carbon dioxide than a constantly maintained ventilation. See, for example, the effects of simply changing the degree of the artificial ventilation on the acidity of the arterial and venous blood, figure 5. The change is prolonged and extensive. Compared with the increase in pulmonary ventilation in figure 3 the increase in artificial ventilation in figure 5 is small. This effect of increased ventilation must then be added to the chemical effect of liberation of alkali on the reduction or lowered oxidation of hemoglobin on the administration of low oxygen. A highly alkalinized blood is in equilibrium with a low alveolar carbon dioxide tension.

Granting the greater elimination of carbon dioxide during the admin-

istration of low oxygen by normal ventilation, the distinct acid overshooting on the readministration of low oxygen by artificial ventilation is attributable to conditions favorable for such change, for the acid which would have left the body as a result of augmented ventilation is present to react with the oxygenated or acidified blood on readministration of room air.

This acid overshooting (in figs. 5, 6, 7 and 8) undoubtedly is in part a contribution of a broken coördination of the dual function of hemoglobin. Continuous records of expired gases support this statement (McGinty

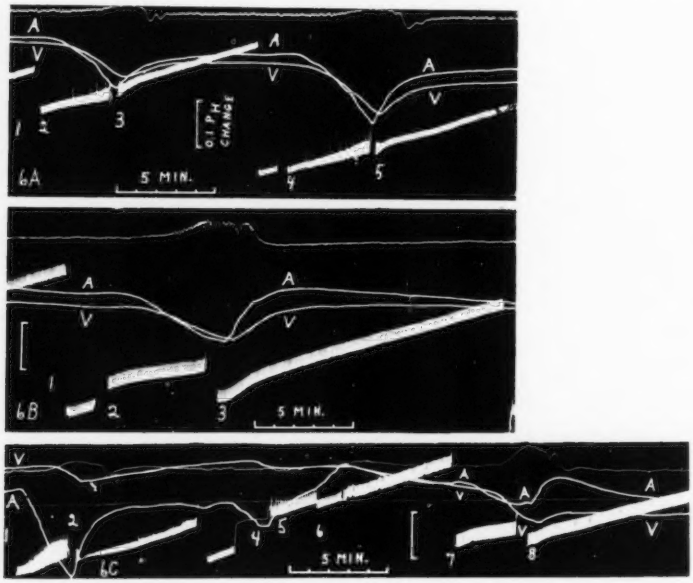
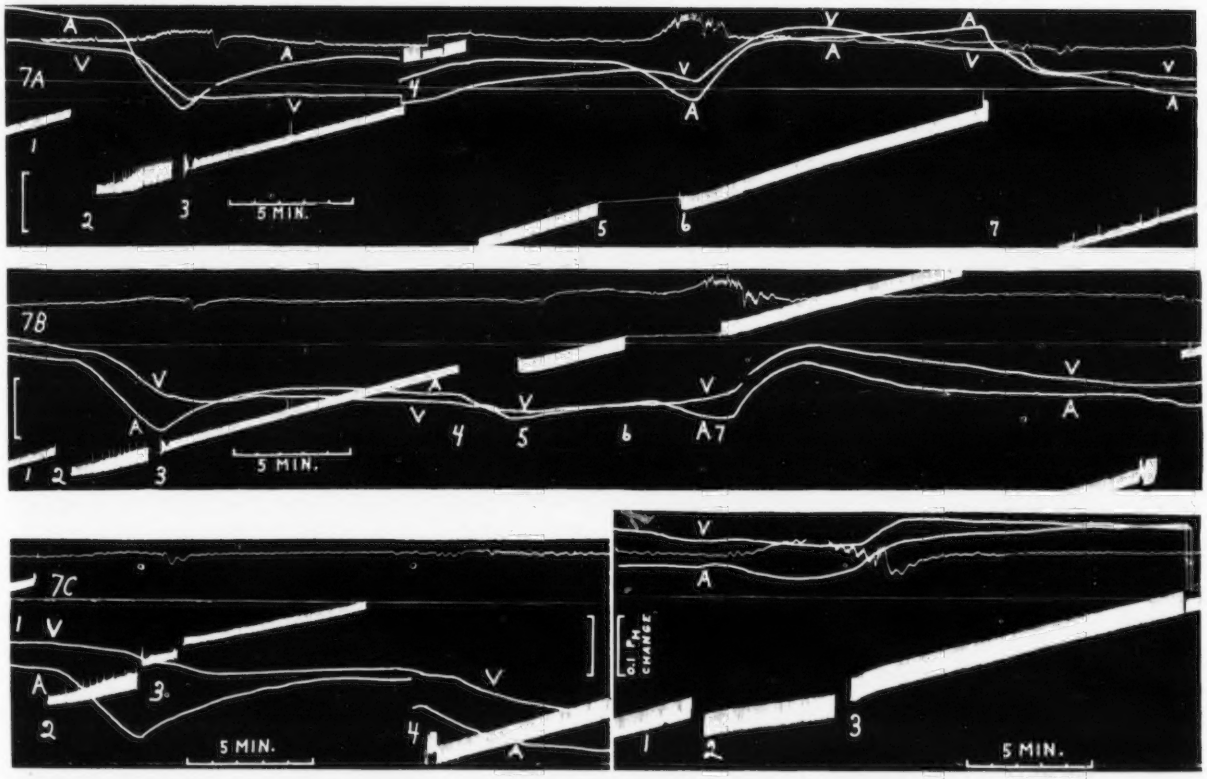


Fig. 6A. Effects of temporary administration of low oxygen by normal ventilation: 6B by artificial ventilation, and 6C by normal and artificial ventilation.

and Gesell, 1927). When low oxygen is temporarily administered by constant artificial ventilation the total amount of carbon dioxide eliminated falls off at once, indicating the importance of oxidation of hemoglobin in liberating the carbon dioxide of the blood into the alveolar air. The blood returns unsaturated to the tissues and as a consequence less oxygen and less acid carrier is liberated at the tissues. Acid tends to accumulate in the blood and tissues. On readministration of room air the conditions are favorable for the elimination of a large amount of carbon dioxide. Oxidations temporarily increase above normal. The accumulated acid



Figs. 7A, B and C. Effects of temporary administration of low oxygen by normal and artificial ventilation

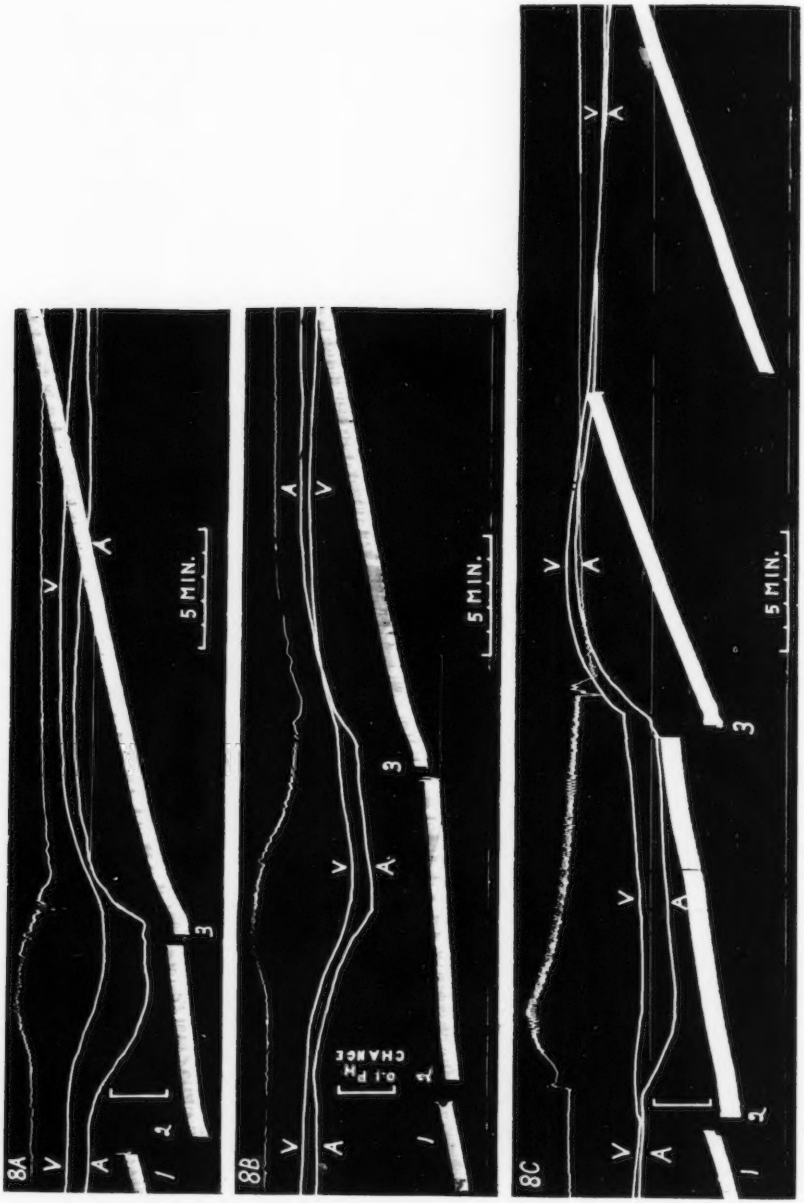


Fig. 8. Effects of more prolonged administration of low oxygen by artificial ventilation

of the blood is driven out by oxidation of the hemoglobin. The continuous record of the expired gases show carbon dioxide elimination considerably above normal. The overshooting of the acid records then correspond to the overshooting of the expired carbon dioxide. The acid overshooting is obviously a combination of two factors: acidified blood (oxidized hemoglobin) in equilibrium with a high alveolar carbon dioxide tension. The absence of sharp overshooting after temporary administration of low oxygen by normal ventilation is, therefore, attributable to the augmented ventilation during the period of low oxygen.

A fairer measure of the acid effects of low oxygen and of the alkaline effects of normal ventilatory response is obtainable when the gaseous mixtures are administered normally and artificially in the same animal. Such observations appear in figures 6 and 7. Figures 6A and B are from one animal, 6C from another, and 7A, B and C from a third. A double pneumo-thorax is established by inserting on each side of the chest a brass tube 1 inch in diameter provided with a stopper and tube for exhausting the chest for subsequent normal ventilation. Pneumo-thorax may again be established by removing the stoppers. Alternate observations may thus be made with normal and artificial ventilation.

In figure 6A the administration of room air by normal ventilation is temporarily interrupted at *2–3*, and again at *4–5* by the administration of a 5 per cent mixture of oxygen. Ventilation and blood pressure are increased. The arterial and venous blood swing sharply alkaline and on readministration of room air return to normal acid values. The greater alkaline effect on the blood of the second administration corresponds with the longer administration. Compare next the effects of low oxygen given by artificial ventilation in figure 6B. Though the duration of administration of low oxygen is longer than either of the preceding observations, the change in alkalinity is smaller. The pronounced acid overshooting appearing on readministration of room air corresponds.

In figure 6 C the animal responds vigorously to low oxygen, as seen in the respiratory record. The change in alkalinity of the arterial blood is accordingly abrupt. At *2* room air is administered. As usual, with short administration of low oxygen there is no acid overshooting. The next observation begins at *7*, where low oxygen is administered by artificial ventilation. The intervening irregularities correspond to maneuvers in changing from natural to artificial ventilation. At *4* the animal receives room air direct—still with natural ventilation. The shortening of the dead space leads to a greater alkalinity of the blood. At *5* artificial ventilation is administered with the chest closed, and at *6* the stoppers are removed. The collapse of the lungs is seen in the record. The increasing acidity of the blood indicates that the ventilation is insufficient. At *6* it is increased with a resulting alkaline change. At *7* low oxygen is ad-

ministered, and at *S* the administration of room air leads to sharp acid overshooting. In figures 7A, B and C the alternate administration of low oxygen by natural and artificial ventilation is shown again. The results are as striking as in the preceding records.

It is not the purpose of this paper to deal with variations in the response to low oxygen, examples of which appear in figure 7. Such details require information which is as yet not available. The volume-flow of blood, for example, is an exceedingly important factor in determining blood acidity (Bald, 1927; Hertzman and Gesell, 1927). It will only be pointed out that in the second observation of figure 7C artificial pulmonary ventilation was considerably greater than in figure 7A and B. The alkaline effect of the change in ventilation is shown at *4*, where the change from normal ventilation to artificial ventilation is made. The excess ventilation may account for the relatively small alkaline effect of the administration of low oxygen. This is upheld on a comparison of figure 8C, in which ventilation was markedly increased, with the two preceding figures, 8A and B.

The marked acid overshooting common to all observations with artificial ventilation and the absence of overshooting with normal ventilation can leave no question as to the two effects which low oxygen tends to exert when administered by normal ventilation: increased alkalinity of the blood due to the liberation of base and washing out of carbon dioxide from excessive ventilation, and an increased acidity of the blood resulting from accession of fixed acid from impaired oxidations in the tissues. Whether or not the tissues will turn acid with low oxygen will then be determined by the resultant of these two effects.

With short administration of low oxygen by artificial ventilation the arterial and venous blood most frequently show only the alkaline change but with more prolonged or severe administration the blood may slowly turn more acid (see figs. 8A, B and C). In figure 8A the administration of low oxygen (4 per cent) is relatively short and the acid increase correspondingly small, but in figure 8B, where the administration is considerably longer, the delayed increase in acidity is very appreciable. The same is seen in figure 8C. In figure 8C the initial alkaline effect of low oxygen is not as great as in 8A and B, due, perhaps, to the markedly greater ventilation that obtains in this record.

From figures 8 and the preceding records we conclude that the initial alkaline change of the blood is deceptive with respect to the changes in acidity in the tissues. The initial increased alkalinity of the blood presumably is associated with a synchronous increase in acidity of the tissues. The change in the tissues, however, is indicated only after the lapse of several minutes. The delayed increased acidity of the blood is conceivably accounted for by the saturation of base liberated by reduction of

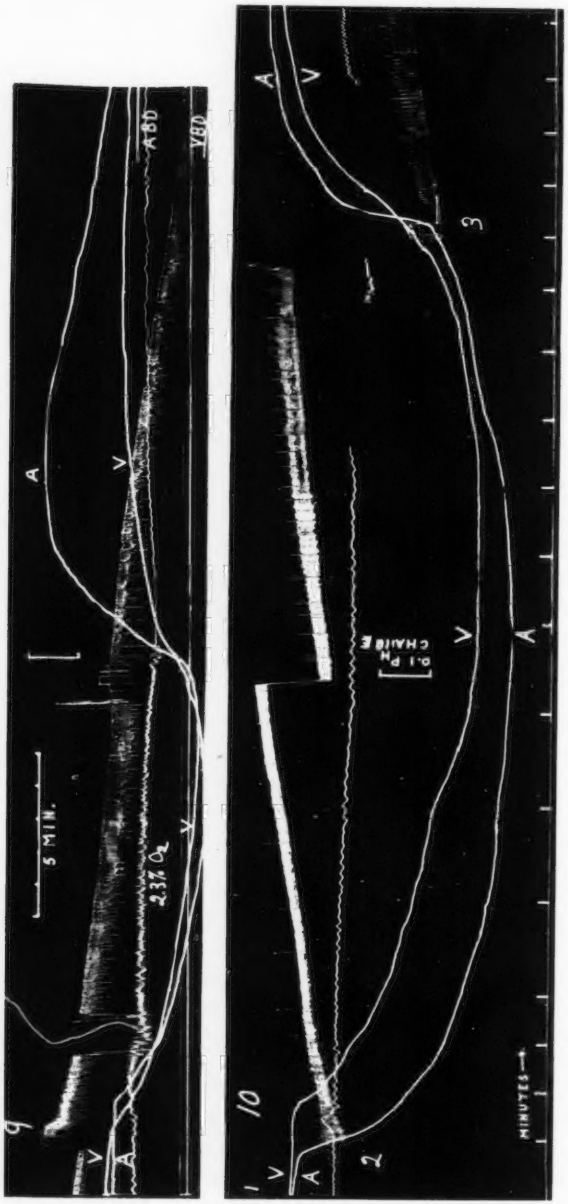
oxyhemoglobin with the carbon dioxide coming from the tissues, and the diffusion of lactic acid from the tissues into the blood stream. The fact that prolonged administration of low oxygen leads to a prolonged increased acidity of the blood after the readministration of room air indicates that fixed acid from the tissues has reduced the buffer base of the blood and contributed to this effect.

In this connection we call attention to the effects of prolonged administration of low oxygen on the elimination of carbon dioxide (McGinty and Gesell, 1927). The first effect is a progressive reduction followed by a delayed increase in elimination approaching normal. This appears related to the delayed increase in acidity of the blood under like conditions, and conceivably is due in part to a displacement of carbon dioxide in the blood and tissues by fixed acids.

Whether the tissues will also turn acid when low oxygen is administered by normal ventilation is, of course, a crucial point in the theory of acid control of ventilation. The prolonged increased alkalinity of the arterial and venous blood during the early stages of administration speaks to the contrary, as, for example, in figure 2B. Yet later when room air is readministered the acidity of the arterial and venous blood rises above the initial value. This has been explained by the passage of fixed acids from the tissues into the blood stream reducing the buffer base. Granting a similar reduction in tissue buffer base, an increase in tissue acidity is most probable.

A surer indication of increasing tissue acidity on the administration of low oxygen by normal ventilation is found in figures 9 and 10. Despite a progressively increasing pulmonary ventilation the rate of increased alkalinity diminishes and ultimately gives way to increasing acidity. Such examples suggest that acid was forming at a faster rate than its removal. The results agree with those of Koehler, Brunquist and Loevenhart (1923). The final high acid values following on the readministration of room air support the inference.

Discussion. Granting that these experiments indicate increased intracellular acidity with the administration of low oxygen and the reverse on readministration of room air, the significance of intracellular acidity in the control of pulmonary ventilation is definitely supported. On the other hand, assuming that the properties of the fluid on the other side of the cell membrane (the immediate cell environment) may be a modifying factor in determining the nature of the discharge of the respiratory neurones, an effort to learn more accurately of the changes occurring in the environment is desirable. Since the environment lies between the blood and the intracellular fluids it is influenced by both. The chemical changes of the environment will then be a resultant of the chemical changes occurring in the blood and in the intracellular fluids and of the restraints offered to chemical exchange through the capillary walls and through the cell



Figs. 9 and 10. Effects of prolonged administration of low oxygen by normal ventilation

membrane. On the administration of low oxygen the immediate environment is then subjected to the influence of increased alkalinity of the blood on the one hand and to the increased acidity of the intracellular fluids on the other hand. A preliminary conjecture taking account of the freedom of movement of materials through the capillary wall, as compared with the freedom of movement through the semipermeable membrane suggests that low alveolar oxygen may turn the immediate cell environment temporarily more alkaline though it produces the opposite effect on the interior of the metabolising cell. Increased alveolar oxygen by increasing the acidity of the blood and improving the oxidations in the cells would conversely increase the acidity of the environment and decrease the acidity of the cell. The acidity of the environment and of the cell would approach each other. The polarization of the cell membrane and the driving head of pressure for the migration of the hydrogen ion would then be altered. Such changes might explain the changing respiratory response along the lines suggested in the electro-chemical working hypothesis of respiratory control (Gesell, 1926), according to which increasing alkalinity of the external environment accelerates ventilation. Considered by themselves the experiments on the effects of low alveolar oxygen suggest the possibility of respiratory stimulation rather than depression by increased alkalinity of the blood and of the immediate environment. Considered along with other types of experiments the probability becomes more uncertain. Obviously a decision on the relative importance of these factors in respiratory control must remain in abeyance until further data are accumulated.

SUMMARY AND CONCLUSIONS

The effects of administration of gaseous mixtures low in oxygen on the acidity of the circulating arterial and venous blood were studied with the manganese dioxide electrode. The results were checked with the hydrogen electrode and agreement was established for the conditions of our experiments.

Temporary administration of low oxygen by normal ventilation elicited increased pulmonary ventilation and increased alkalinity of the arterial and venous blood. On the readministration of room air the arterial and venous blood returned approximately to their normal acid values and ventilation subsided.

Temporary administration of low oxygen by artificial ventilation equal to the preceding ventilation with room air increased the alkalinity of the arterial and venous blood. On readministration of room air the arterial and venous blood temporarily turned more acid than normal.

A comparison of alternate administration of low oxygen by normal and artificial ventilation of relatively short but equal duration on the same animal

showed a greater increase in alkalinity of the blood with normal ventilation. On readministration of room air the acid overshooting invariably present with artificial ventilation was usually missing with normal ventilation.

Prolonged administration of low oxygen by artificial ventilation elicited a slowly developing delayed acidity of the arterial and venous blood following the initial increase in alkalinity.

Prolonged administration of low oxygen by normal ventilation elicited a greater initial alkalinity of the blood and a more delayed acid change.

It is concluded that the changes in blood acidity resulting from variations in alveolar oxygen pressure during constant artificial ventilation are a function of the state of oxidation of the hemoglobin and the degree of coördination of the dual function of hemoglobin—the increased alkalinity with low alveolar oxygen being a result of the liberation of alkali by the hemoglobin of the blood.

The greater increased alkalinity of the blood resulting from the administration of low oxygen by normal ventilation is a combined function of the state of oxidation and the increased pulmonary ventilation.

The acid overshooting on readministration of room air during constant artificial ventilation is an index of the efficacy of normally augmented ventilation during the administration of low oxygen.

The delayed acid change of the blood during prolonged administration of low oxygen indicates the acid effects of low oxygen and the deceptiveness of the initial alkaline change of the blood as an index to tissue acidity. It was suggested that the acid effects of low alveolar oxygen are a result of disturbed oxidation in the tissues and to a broken coördination of the dual function of hemoglobin.

Administration of low oxygen by normal ventilation may then elicit an immediate acid effect upon the tissues, which is counteracted by the alkaline effect of increased ventilation. The resultant of these two effects presumably determines to a large extent the effect of alveolar oxygen on tissue acidity.

The ultimate increased acidity of the arterial and venous blood during prolonged administration of low oxygen by normal ventilation indicates that the alkaline effects of increased pulmonary ventilation may not keep pace with the altered acid metabolism. Acids may form at a faster rate than their removal.

The results support the theory that augmented ventilation elicited by low alveolar oxygen is associated with increased intracellular acidity; that subsequent depression of ventilation from the administration of room air is associated with a decreased intracellular acidity.

It was suggested that the immediate cell environment may turn more alkaline with the administration of low oxygen and again swing acid on

the readministration of room air. The significance of such changes in the control of ventilation is briefly discussed.

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THE REGULATION OF RESPIRATION

XII. The Vagal Reflex Control of the Respiratory Movements of the Isolated Head. Peripheral Mechanical and Peripheral Chemical Factors

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Though the literature on respiration is replete with studies on the central chemical regulation of respiration, the possibility of a peripheral chemical control has generally been overlooked. Recently, however, Heymans and his collaborators (1925, 1925a, 1925b, 1926a, 1926b, 1926c, 1926d) have suggested that chemical changes, such as anoxemia and rising CO_2 tension of the vagal endings, as well as mechanical changes, such as stretching of the vagal endings, occurring in the cardio-pulmonary system, may originate afferent vagal impulses importantly influencing the bulbar circulatory and respiratory centers. These impulses may be either inhibitory or stimulating causing corresponding effects in the motor part of the reflex thus set in motion.

The experimental method on which these conclusions are based (Heymans and Ladon, 1925) consisted in differentiating between purely central and purely peripheral stimuli by severing all connections, except the vagi, between the head and trunk of an animal. The head was kept alive, independent of the trunk, by intercalating it in the circulation of a second animal. Artificial ventilation served the trunk. The activity of the respiratory center was followed by recording laryngeal movements.

The conclusions of Heymans incited us to obtain first hand information on the relative importance of central chemical, peripheral mechanical and peripheral chemical control of pulmonary ventilation. Though our results are similar in certain respects, the conditions which we have provided have led to sufficient variation to warrant their description. In comparing our results with those of Heymans and his associates it should be borne in mind that our experiments are very limited in number and that the present report is preliminary.

Method. Two dogs, under morphine-urethane narcosis, were prepared for a carotid to carotid and jugular to jugular anastomosis, as in Heymans' method. (The donor should be preferably somewhat larger

than the receiver.) Heparin was injected into the donor to prevent blood coagulation. Artificial ventilation was supplied to the receiver's trunk by means of an electrically driven pump, placed in circuit with rebreathing tanks. The smooth operation of the pump insured a smooth gradual inflation of the lungs similar to that of quiet inspiration. The lungs deflated passively against a minimal resistance. Ventilation could be kept constant, suspended, or changed as desired. Gases of the composition desired were administered from the rebreathing tanks.

Having completed the preparation of both the donor and receiver the neck of the receiver, with the exclusion of the vagi, is thoroughly crushed with a powerful vise (fig. 1) at the level of the 4th or 5th cervical vertebra During crushing of the neck, the mean blood pressure in the trunk falls to a low level (30 to 70 mm. Hg), which is maintained with slight variation throughout the experiment. The trunk presents the usual picture of cord transection. It is, therefore, suggested that the malnourishment of the trunk which probably exists may color the interpretation of our results, yet similar pressures in the intact animal seldom interfere with the usual respiratory response to newly established conditions.

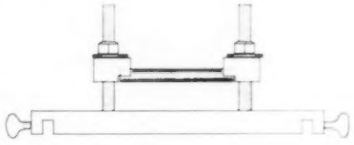
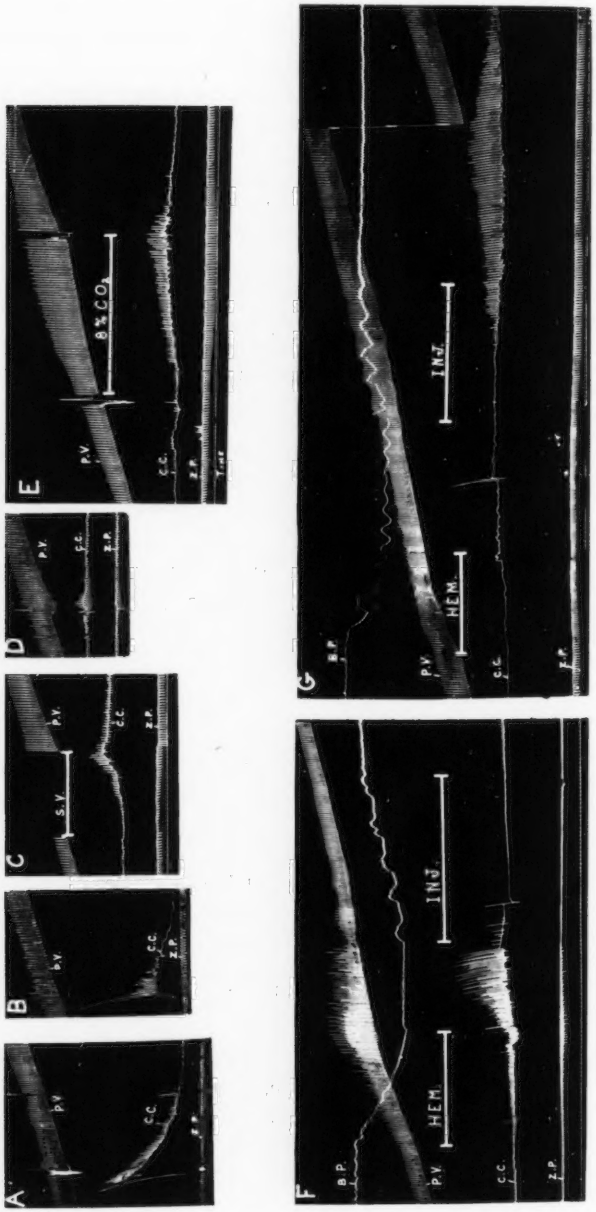


Fig. 1. Vise for crushing a dog's neck. The lower horizontal bar clamps on the operating board. The upper horizontal bar is a steel rod. The animal's neck is placed between the two bars and crushed by turning the nuts with a wrench.

In casting about for a means of recording the activity of the respiratory center of the isolated head, we noticed that in the intact animal, the cricothyroid muscle contracted with each respiratory movement of the lungs, causing a slight movement of the cricoid cartilage. This movement can be conveniently recorded by a pulley and lever arrangement. As a rule, these movements indicate fairly closely the rate and extent of pulmonary ventilation. Certain exceptions occur which will be pointed out below.

RESPIRATORY SIGNIFICANCE OF THE MOVEMENTS OF THE CRICOID CARTILAGE. Control experiments were carried out on the intact animal to determine to what extent we could use the movements of the cricoid cartilage as indicative of the activity of the respiratory center during various types of stimulation and inhibition. In these control experiments the motor and sensory innervation of the larynx and trachea was intact. Therefore, the movement of the cartilage is the resultant of contraction of the entire group of laryngeal muscles, though in ordinary quiet respiration the crico-thyroid muscle alone seemed to be active.[1]

[1] Changes in tension of the vocal cords with pulmonary movements do not appear to be transmitted to any important extent to the cricoid cartilage.


A
P.V.
C.C.
Z.P.
B
P.V.
C.C.
Z.P.
C
S.V.
P.V.
C.C.
Z.P.
D
P.V.
C.C.
Z.P.
E
P.V.
8% CO2
C.C.
Z.P.
F
B.P.
P.V.
HEM.
INJ.
C.C.
Z.P.
G
B.P.
P.V.
HEM.
INJ.
C.C.
Z.P.

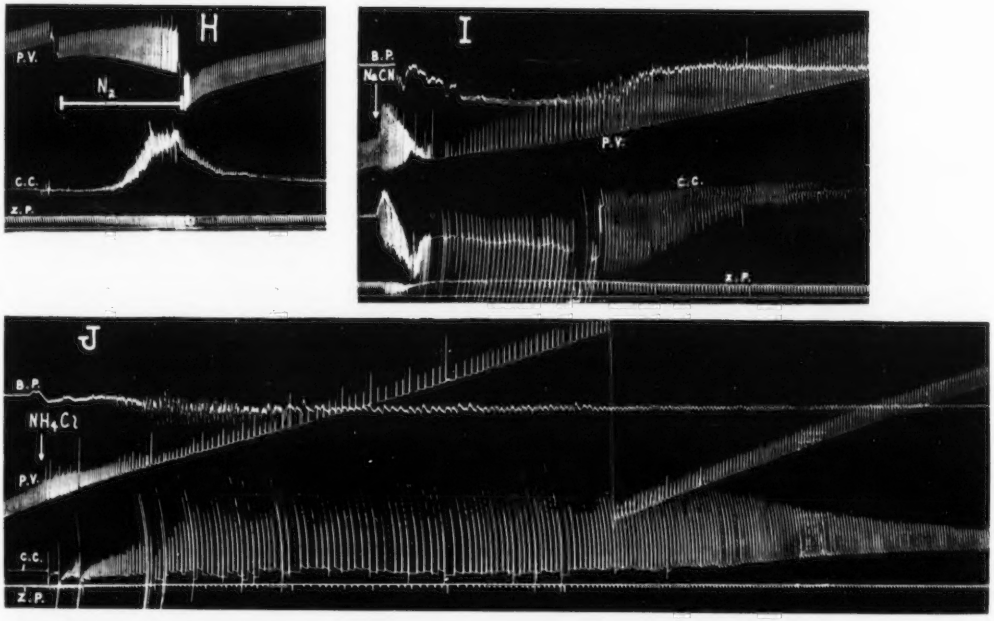


Fig. 2. Comparison of movements of cricoid cartilage, *c.c.*, with pulmonary ventilation, *p.v.*, and movements of xiphoid process, *z.p.* *B.P.* = blood pressure.

Record A: strong stimulation of sensory nerve. Record B: weak stimulation of sensory nerve. Record C: suspended ventilation. Record D: intravenous injection of $NaHCO_3$. Record E: administration of 8 per cent CO_2 in room air. Record F: hemorrhage followed by injection of a 6 per cent dextrose solution instead of blood. Record H: administration of N_2—heavy bar represents period of administration. Record I: intravenous injection of NaCN at arrow. Record J: intravenous injection of NH_4Cl at arrow.

In the crossed-circulation experiments the recurrent nerve is destroyed, so that, of the laryngeal group, the crico-thyroid contractions alone are recorded. In both groups of experiments, contractions of the muscles further cephalad, particularly the depressors of the jaw, are also recorded on the cricoid records, especially during acute respiratory distress.

1. *Stimulation of sensory nerves.* This had a more pronounced effect on the movements of the cricoid cartilage than on pulmonary ventilation. The threshold stimulus seems to be lower. The typical effect (fig. 2A and B) consists of a sharp increase in tonus with strong contractions extensive in character during stimulation. After stimulation, the contractions slowly decrease in height, with a gradual decrease in tonus.

2. *Suspended ventilation.* The gradually increasing activity of the respiratory centre is well illustrated in the cricoid record (fig. 2C).

3. *$NaHCO_3$.* The respiratory stimulating effect is illustrated in the cricoid record (fig. 2D).

4. *CO_2.* The increase in pulmonary ventilation is accompanied by a corresponding increase in tonus and contractions of the laryngeal muscles, the maximum occurring with that of pulmonary ventilation (fig. 2E). The increase in tonus does not always occur, even though the contractions do increase. In a few instances, the agreement between the pulmonary ventilation and the cricoid records was poor. In one case there was a very slight late increase in the cricoid movements; in another instance the maximum of the cricoid movements occurred early before pulmonary ventilation reached its maximum. In this case the cricoid movements were increasing before the administration of CO_2 so that it is uncertain how great was the effect of CO_2. In all cases, however, the stimulation to the respiratory center is, at least qualitatively, reflected in the cricoid records.

5. *Hemorrhage followed by injection of the withdrawn blood.* Usually, the cricoid and pulmonary ventilation records show agreement in this procedure. Occasionally, late after injection of the withdrawn blood the cricoid movements were exaggerated.

6. *Oxygen-want.* A good agreement existed between the cricoid and pulmonary ventilation records (fig. 2H).

7. *NaCN.* The cricoid record reflects as a whole the activity of the respiratory center (fig. 2I), though the cricoid movements are more exaggerated than what one would expect from the pulmonary ventilation record. This is especially noticeable during the period of respiratory depression.

8. *NH_4Cl.* On the injection of this substance, there was invariably a marked disagreement between the pulmonary ventilation and cricoid records. With the small doses the disagreement was not as pronounced as with the larger doses, though yet very apparent. There may be a very

slight effect on respiration and a comparatively enormous increase in the cricoid movements. With the larger doses, there is a tremendous increase in extent of the cricoid movements at the time that pulmonary ventilation

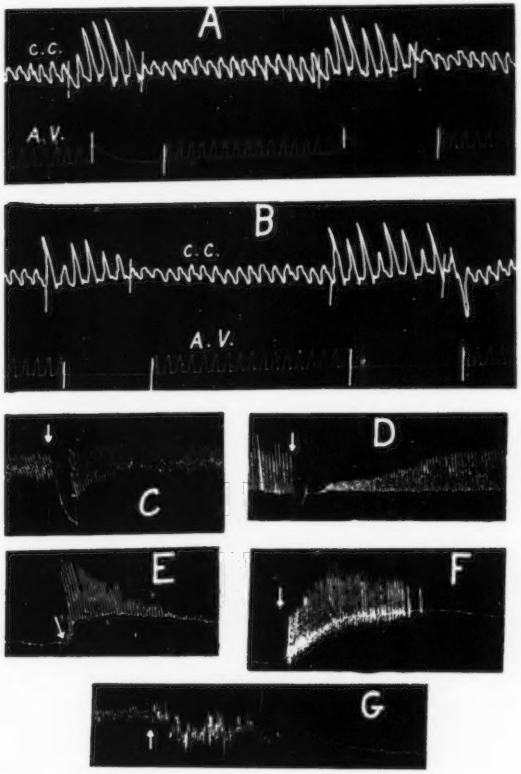
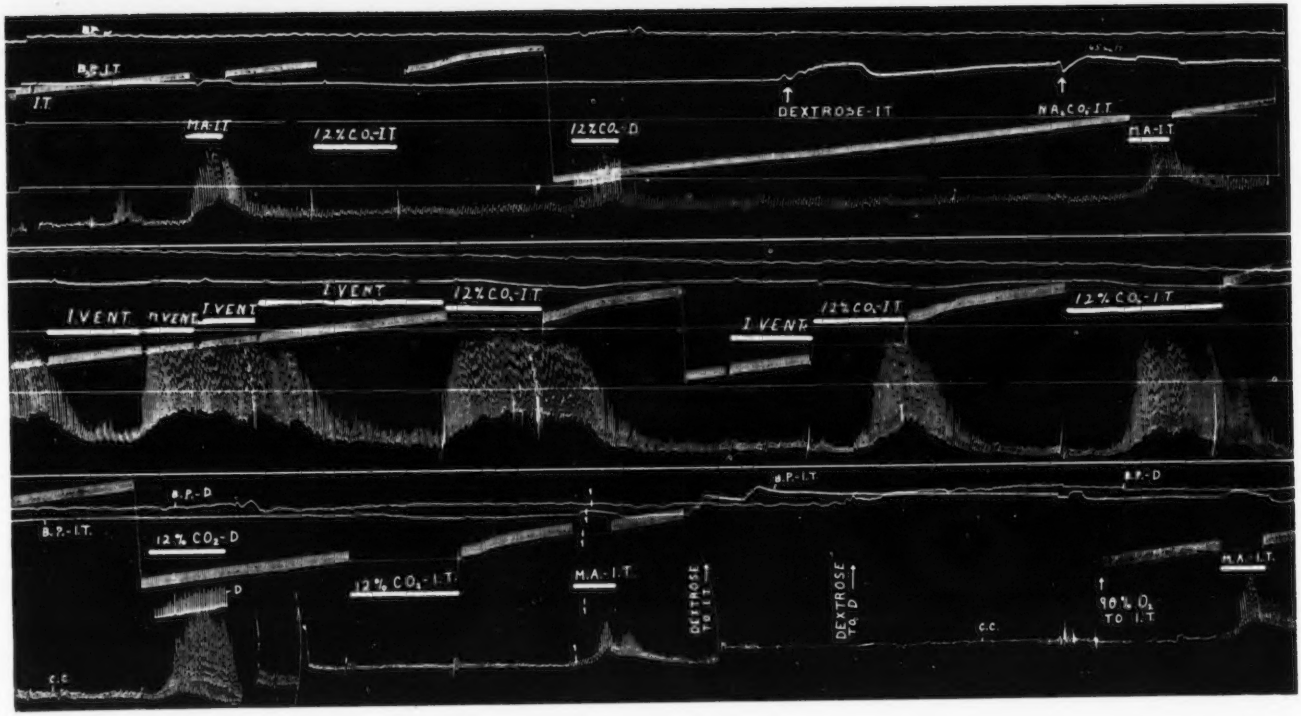


Fig. 3. Records A and B: effect of suspended ventilation, *A.V.*, in isolated trunk on movements of the cricoid cartilage, *c.c.*, of the isolated head. Record A: stopping ventilation at end of inspiration. Record B: stopping ventilation at end of expiration. Cricoid record retouched. Records, C, D and E: effects of faradic stimulation of the vagus on the movements of the cricoid cartilage of the isolated head. Records F and G: effects of removal (record F) from and application (record G) of cold blocks to the vagi on the movements of the cricoid cartilage of the isolated head.

is less than normal. Then as the ventilation again improves the cricoid movements gradually decrease towards normal, this decrease being associated with an increase in respiratory efficiency.


B.P.
B.P. I.T.
I.T.
M.A.-I.T.
12% CO2-I.T.
12% CO2-D
DEXTROSE-I.T.
NA2CO3 I.T.
M.A.-I.T.
I.VENT.
I.VENT.
I.VENT.
12% CO2-I.T.
I.VENT.
12% CO2-I.T.
12% CO2-I.T.
B.P.-D.
B.P.-I.T.
12% CO2-D
12% CO2-I.T.
M.A.-I.T.
DEXTROSE TO I.T.
DEXTROSE TO D.
90% O2 TO I.T.
M.A.-I.T.
C.C.

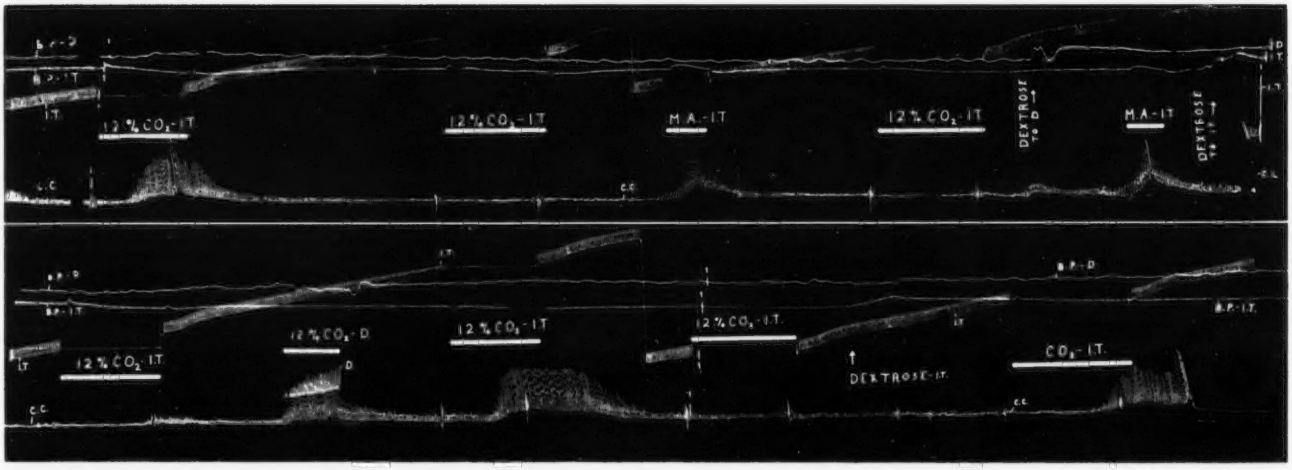


Fig. 4. (The five records give a continuous record of one day's experiment.) A comparison of peripheral chemical and peripheral mechanical elements in originating vagal afferent impulses affecting the respiratory center of the isolated head. *B.P.D.* = blood pressure of donor. *B.P.I.T.* = blood pressure of isolated trunk. *I.T.* = pulmonary ventilation of isolated trunk. *C.C.* = movements of cricoid cartilage of isolated head. *Vent.* = ventilation; *I* = increased; *N* = normal. *M.A.* = suspended ventilation. Type procedure: 12 per cent CO_2-I.T. refers to administration of 12 per cent CO_2 in room air to the isolated trunk, while 12 per cent CO_2-D similarly refers to administration of 12 per cent CO_2 to the donor. The separation of the records is indicated by the broad horizontal white lines. Read from left to right.

These control experiments indicate that the records of the cricoid movements give a qualitative picture of the activity of the respiratory center. Quantitative significance can not be attached to them. Because of the great disagreement existing in the case of NH_4Cl, the effects of this substance are not reported in the description of the following experiments.

THE PERIPHERAL REFLEX CONTROL OF THE RESPIRATORY CENTER OF THE ISOLATED HEAD. *A. Mechanical elements.* The influence of the state of tension of the lungs and of the movement of air in the larynx and trachea on the activity of the respiratory center has been repeatedly emphasized in the literature. Hering and Breuer, Gad, Head, Meek and others have called attention to the mechanical stimulation of the end-organs of the vagi in the lungs. The mechanical effect is so apparent that the apnea resulting from excessive mechanical stimulation has been called "vagal apnea." Meek (1924) states that the mechanical effect could be elicited even in the presence of a high CO_2 content in the inspired air.

Our own experiments on the whole support the significance of mechanical influence in eliciting reflex vagal effects on the activity of the respiratory center of the isolated head and in this respect our results differ from those described by Heymans. When artificial ventilation of the isolated trunk is suspended, there frequently results an immediate increase in the movements of the cricoid cartilage of the isolated head (fig. 3). This is probably the result of the cessation of stimuli (from alternate distention and collapse of the lungs) to the vagal nerve endings, causing a removal, at least partial, of the vagal inhibitory effect on the respiratory center. The presence of 90 per cent oxygen in the lungs did not modify the development of this effect. A rising alveolar CO_2 tension, similarly, does not appear to be the cause since the effects are so immediate.

The instant at which the cricoid movements first increase following cessation of artificial ventilation depends on whether the pump is stopped at the end of inspiration or at the end of expiration. In the latter case (fig. 3B) there is a sharp increase in the cricoid movements with the very next contraction. In the former case (fig. 3A), the increase is delayed one contraction. This would seem to indicate that a state of tension in the lung tissue is a primary element in the stimulus to the vagus endings, though this does not imply that periodicity in the development of a state of tension or changing tension is unessential to the mechanical stimulation of the vagal endings.

Similar mechanical effects may be observed on changing the depth of ventilation of the isolated trunk. There are corresponding immediate changes in the cricoid movements (fig. 4). Increased depth of ventilation results in decreased cricoid movements. Decreased depth of ventilation has the opposite effect.

Heymans and Heymans (1926a) found that during suspension of artificial ventilation of the trunk the respiratory movements of the head remain at first unchanged in frequency and amplitude, then finally become more and more frequent and extensive to the degree that the trunk is asphyxiated. This is contrary to our own findings. We have been able to distinguish, in all our experiments, the immediate mechanical effects both on the cessation and the beginning of artificial ventilation. So constant (in comparison with the inconstant chemical effects) are the mechanical effects that we have employed them together with faradic stimulation of the vagi as a means of determining, during the course of an experiment, the integrity of the central and peripheral connections of the vagi.

The importance of mechanical effects is further illustrated by the observation that usually the movements of the cricoid cartilage are synchronous with the pump supplying the isolated trunk with air, each contraction of the crico-thyroid muscle just following expiration by the lungs of the trunk.

These observations, emphasizing the importance of mechanical stimulation of the pulmonary end organs of the vagi, indicate that the smooth physiological coördination of pulmonary ventilation with the respiratory needs of the animal is to an important extent effected through a control initiated by mechanical changes in the lungs. This, however, does not rule out the possibility of subsidiary yet important influences being exerted by peripheral chemical changes which are discussed below.

B. *Faradic stimulation of the vagi.* An analysis of the rôle of the vagi in influencing the respiratory movements of the head emphasizes the difficulty of a detailed interpretation of vagal respiratory reflexes arising from chemical changes in the lungs and circulation of the trunk. The literature on the rôle of the vagi in respiration by no means offers a detailed working schema. The fact that excitant impulses, as well as the well-known inhibitory impulses, may be conducted by the vagi to the respiratory center complicates the situation. In our own experiments the central respiratory effects from faradic stimulation of the vagi were by no means constant; on the contrary a considerable variability obtained in the movements of the cricoid cartilage. Figure 3C to G illustrates this. Either stimulation or inhibition of the respiratory movements of the head may be obtained. The most frequent result was inhibition during the actual period of faradic stimulation, followed by a period of respiration above normal.

C. *Vagal respiratory reflexes having apparently an origin in chemical changes in the trunk.* Heymans and Heymans (1926a) observed that the administration of CO_2 to the isolated trunk was particularly effective in eliciting a respiratory response in the isolated head. They conclude that

the frequency and amplitude of the activity of the respiratory center are under the influence of the CO_2 tension of the peripheral blood and alveolar air, diminution of CO_2 through hyperventilation inhibiting (reflex apnea), and augmentation of CO_2 through asphyxia, exciting the respiratory center (reflex hyperpnea). They further state that the vagal respiratory tonus is not mechanical but humoral in origin—modifying the statement by the following: "cette conclusion n'exclut pas que d'autres excitations reflexes, de nature chimique on physique, peuvent également influencer la respiration."

We have, similarly, found that the administration of CO_2 to the isolated trunk is apparently effective in eliciting a respiratory response in the head. Frequently no response is obtained. Figure 4 illustrates how the response may vary during the course of an experiment. This figure gives a continuous record of one day's experiment. A detailed consideration of it reveals some interesting facts. Beginning with the first mechanical asphyxia of the trunk, the head gives the usual immediate response to this procedure. The cricoid movements increase during the asphyxia, decreasing again when the pump starts. Following shortly on this, 12 per cent CO_2 was administered to the trunk. That the CO_2 reached the trunk is indicated by the fall in blood pressure in the trunk and also by the increase in the basal metabolism gradient (due to absorption of CO_2 by the soda-lime cartridge) when room air was again administered. (This was true also for all subsequent trials with CO_2 to the trunk.) Yet there is no indication of a reflex effect on the respiratory center of the head, though the momentary cessation of the pump at the moment of shifting from one tank to the other is marked on the cricoid record, showing the effectiveness of the mechanical changes in eliciting a reflex response. Following CO_2 to the trunk, 12 per cent CO_2 was administered to the donor. The typical central effect appears—respiratory stimulation increasing with the duration of the administration. These three results taken together seem to indicate the relative importance of central chemical changes, peripheral mechanical and peripheral chemical changes in the control of respiration under the conditions supplied. The peripheral chemical changes seemed ineffective in this particular case. A little later, the response to mechanical asphyxia is again elicited. Changing the ventilation of the trunk also had a pronounced effect on the respiratory activity of the head, as shown in the following observation,—increasing the ventilation inhibiting the respiratory center and decreasing the ventilation having the opposite effect. Is the origin of this reflex mechanical or chemical? The mechanical elements seem predominant since the sharp increase in the cricoid movements on decreasing the ventilation is too immediate to be due to chemical changes. The further increase in the response of the head does not necessarily indicate that it is due

to a chemical change in the trunk. It may be that the response to mechanical changes gradually builds up, reaching its maximum some time after the initiation of the change. One cannot neglect such a possibility in interpreting the results from inducing chemical changes in the trunk. This is particularly evident in the next procedure, administration of 12 per cent CO_2 to the trunk. The abrupt increase in the cricoid movements on changing gases indicates that at this moment the preparation was particularly sensitive to mechanical changes. We are uncertain as to what significance should be attached to CO_2 in accounting for the marked stimulation. The next two administrations of CO_2 to the trunk elicited a delayed gradually increasing respiratory activity of the head, of the type that one would expect to get from a reflex stimulation having its origin in an increasing CO_2 tension in the periphery. Subsequent administration of CO_2 to the donor resulted in the usual central stimulation, while CO_2 to the trunk immediately following had no effect, though the head was still sensitive to the mechanical changes, as indicated by the response to suspended ventilation, whether the lungs are filled with room air or 90 per cent oxygen. The rest of the experiment continues in a similar manner. The response to the last CO_2 administration to the trunk is peculiar in the abrupt cessation of the cricoid movements some time after the readministration of room air. We see no reason for this.

Neither have we had the same success as Heymans and Heymans (1926a) in eliciting a delayed respiratory stimulation of the head from oxygen want (administration of nitrogen). We have been able to obtain stimulation by inducing anoxemia in one experiment where hyperpnea occurred after the death of the trunk following the administration of nitrogen. In contrast—central anoxemia induced by administering a low percentage of oxygen to the donor produced stimulation of the isolated head and of the donor.

The injection of sodium cyanide into the trunk gave more positive results but these were inconstant. In some cases no effects were obtained. Central administration to the isolated head was invariably followed by increased respiration.

The inhibiting and stimulating effects of direct administration to the isolated head of Na_2CO_3 and $NaHCO_3$ were missing on injection in the isolated trunk.

Discussion. It would be of interest to know the explanation of the difference between the results of Heymans and ours. The conditions in the experiments of Heymans led to augmented effectiveness of the peripheral chemical factors and depression of the peripheral mechanical factors. The conditions in our experiments have apparently enhanced the effectiveness of the peripheral mechanical factors and failed to bring out clearly the peripheral chemical factors of control. Our experiments

would, therefore, support the view of the significance of the peripheral mechanical factors of the control of pulmonary ventilation. While they can also be said to support the possibility of a normal mechanism of peripheral chemical control, they can hardly be considered by themselves to point to that probability.

SUMMARY

The relative significance of peripheral mechanical and peripheral chemical factors in the vagal reflex control of the activity of the respiratory center of the isolated head has been studied in a preliminary way by means of crossed-circulation experiments.

Effects were registered of mechanical and chemical changes in the isolated trunk, exerted through the intact vagus nerves, on the behavior of the cricoid movements of the corresponding isolated head.

Movements of the cricoid cartilage of the intact animal were previously compared with synchronous changes in pulmonary ventilation. Fairly close correspondence was found.

Suspended artificial ventilation of the isolated trunk almost invariably elicited increased respiratory movements of the isolated head. When ventilation was stopped at the end of expiration the effect was immediate; when stopped at the end of inspiration the effect was delayed one respiratory cycle. The same results were obtained whether the lungs were filled with room air or oxygen at the moment of suspension.

The administration of carbon dioxide, of air low in oxygen, and of sodium cyanide to the isolated trunk elicited on the whole inconstant and delayed reflex stimulation of the respiratory muscles of the isolated head.

Similar administrations to the isolated head via the donor elicited constant and prompt stimulation of respiratory movements in both the isolated head and the donor.

The relative significance of central chemical, of peripheral chemical and of peripheral mechanical factors of respiratory control are briefly discussed.

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THE PULSE RATE OF THE NORMAL RAT[1]

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In connection with other studies the necessity arose for determining as accurately and expeditiously as possible the pulse rate of normal albino rats.

METHODS. To determine this rate directly by mechanical means was proved in certain preliminary studies to be feasible but the method involved either restraining or anesthetizing the rats, both of which procedures cause considerable deviation from the normal. Moreover, anesthesia, as is becoming well known, has a tendency to fix the rate within relatively narrow limits, whereas, in the projected studies it was desired to leave the pulse rate normally labile. The rate was determined by three methods: a, placing a straw on the thorax over the apex of the heart and allowing the straw to actuate a recording lever; b, attaching the end of a transected carotid artery to a recording lever (Lombard method); c, placing a loop of thread under the carotid and attaching it to the writing lever, thus transmitting the tug of the artery. The record reproduced in figure 1 illustrates the ease with which records so obtained can be read.

The method selected for the investigation proper was auscultation of the heart beat by aid of a stethoscope and signaling each beat electrically by means of a key and marker. Throughout most of the experiments a Ford stethoscope was employed with the gutta percha end of the small bell removed. Toward the end it was found that an ordinary glass Y-tube, 4 mm. in diameter, is a more satisfactory receiver than the stethoscope bell in that distinct heart sounds are transmitted while respiratory and skeletal muscle sounds are largely eliminated. In the case of adult rats under quiet conditions and with relatively slow pulse rates, the heart beats could be signaled by any type of simple spring key. In certain cases, however, in which the rate was approximately seven hundred beats per minute, the difficulty of synchronizing contacts and heart beats became great. This difficulty was obviated by the use of a copper yoke held in the fingers and with which the auditor beat "two-four" time. This was moved back and forth to make contacts at both up and down strokes with

[1] Investigation supported by The Memorial Foundation for Neuro-endocrine Research.

a nickeled brass bar conveniently held in a clamp. A Lieb time marker set to register five-second intervals was used. A Becker signal magnet was selected because of its relatively quiet operation. The pulse and time signals were recorded simultaneously by means of an ordinary kymograph. The pulse signals were then counted with the aid of a reading glass. It was realized that the use of a magnetic counter would obviate

TABLE 1

Control determinations of rate of stop watch ticking 300 times per minute

NUMBER OF TRIAL	COUNTS					AVERAGE
1	292	300	299	298	294	298
2	304	290	291	291	276	294
3	300	309	290	300	303	300
4	294	304	320	304	298	304
5	280	314	302	304	302	300
6	297	300	300	300	298	300
7	312	305	312	330	300	310
8	300	330	304	321	215	315
9	303	316	309	315	318	315
10	318	308	309	306	313	310
11	320	300	292	300	318	305
12	304	327	320	312	318	315
13	304	302	300	300	300	300
14	308	300	318	308	312	310
15	304	297	298	297	298	299
16	300	301	318	300	304	305
17	300	300	298	318	300	304
18	300	300	307	300	340	308
19	300	300	301	300	303	301
20	290	306	285	294	300	295
21	300	300	300	300	312	302
Average						304

	per cent
Maximum error of any determination	10
Maximum error of any group	±5
Average error of series	±2.3
Absolute error of total series	+1.3

much drudgery but one could not be obtained before the completion of these experiments.

A check on the accuracy of the method was run throughout the series of experiments (about once for each five pulse determinations) by recording the ticks of a stop-watch (300 per minute) muffled in a towel to simulate as nearly as possible the quality and intensity of the rat's heart beat

(see table 1). This determination proved more difficult, as a matter of fact, than that of the pulse rate because of the similarity in quality between the ticks of the watch and the clicks of the signal magnet, which similarity gave rise to a tendency to react to the wrong sound. Stop-watch counts were tabulated in groups of five, consecutively, to correspond with the method of dealing with the pulse rates of the individual rats. The greatest error in any single stop-watch check was 10 per cent; that for any single group was 5 per cent. The average error for the whole series was 2.3 per cent. The average of the whole series of records was 304 or 1.3 per cent greater than the true value. Assuming the adequacy of the check, therefore, the average pulse rate as herein recorded is 1.3 per cent too great.

Another check was run a few times by recording simultaneously the pulse of an anesthetized rat mechanically and by the auscultatory method. Figure 1 shows the results of a satisfactory check. Discrepancies up to 5 per cent were noted in some cases. These are ascribed to the difficulties

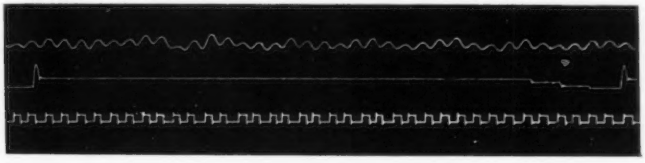

Fig. 1. Showing graphic record of carotid pulse determined mechanically and by the auscultatory method simultaneously. Time, 5 seconds.

under which the check was run, the noises of the signal magnet and the moving kymograph proving unduly distracting to the auditor because of his close proximity. Ordinarily the auditor was stationed about ten feet from the recording set-up and was little disturbed by the noises incident to the recording. It is worthy of emphasis that the auditor, the recorder, and the counter worked independently without interchange of information that might interject a subjective factor.

It was found that the greatest source of error was fatigue of the auditor which tended to introduce unsteadiness in the manipulation of the key. This unsteadiness was readily detected by the recorder, however, and the more unsatisfactory records were discarded at once. The accuracy of the data could have been further enhanced by discarding all periods in which the stop-watch checks showed significant degrees of error, but for the purposes of this research the data are sufficiently accurate as they stand.

The attempt was made throughout the experiments to obtain as nearly as possible a basal pulse rate, *i.e.*, one free from accelerations incident to

movement or tenseness in the subject. This was fairly well achieved by taking advantage of the fact that well-fed rats at a comfortable temperature spend a large part of their time relaxed and half asleep. Groups of about five were placed in small boxes and kept warm by an overhead 40 watt lamp or a reflecting electric heater, giving a temperature in the box of 25 to 30°C. The purpose of having several rats in the box was to exploit the fact that under such conditions individual rats ordinarily pay little attention to being nudged by their fellows, and hence were usually little disturbed by the application of the stethoscope. In most cases, as a matter of fact, there was some movement or change of tenseness in the animals. The maximum deviation from this cause was shown in the case of a rat, successive pulse rates of which for five determinations, were

TABLE 2

Showing the effect of extending the number of determinations on the pulse average

RAT NUMBER	DETERMINATIONS	
	Five	Ten
11	297	292
19	296	301
23	312	315
25	288	290
26	322	328
28	317	322
29	322	320
30	320	323
33	293	295
68	323	297
74	274	281
Average.....	306	306

342, 274, 266, 259, 274, giving an average of 283; in a record showing about mean variability the rates were 305, 309, 330, 310, 325, averaging 316; in the least variable record the values were 322, 320, 318, 316, 308, averaging 317. No very satisfactory correlation in case of these relatively quiet animals could be made between the degree of wakefulness of the rats as reported by the auditor and the actual pulse rate recorded. By taking sufficient time it would have been possible to obtain records under strictly basal conditions. The average of the lowest rate recorded for each of 100 rats was, as a matter of fact, 281 with a mean deviation of ± 18, whereas the average for all values recorded was 305, or 8.5 per cent higher. Ordinarily the pulse was recorded throughout thirty seconds but in some cases twenty, and others, forty seconds was the period employed.

How many determinations were requisite to establish a satisfactory

average pulse rate presented an interesting problem. A series of ten determinations was made during a single experimental period on each of ten animals. These were averaged at the end of five determinations and again at the end of the series. Table 2 shows the results. There was no consistency in the direction of change of average when the larger series was taken. The total average based on five determinations was exactly the same as that based on ten, namely, 306. It appeared then that five determinations gave as satisfactory results as larger numbers, hence

TABLE 3

Pulse rates of 3 groups of 5 rats each, 3, 7 and 20 days old, respectively. All rats were held but were quiet

RAT	AGE	WEIGHT	PULSE (5) COUNTS					AVERAGE
	days	*grams*						
M.	3	8	330	285	285	288	294	296
F.	3	8	244	288	288	288	288	279
M.	3	7.7	318	306	312	330	318	317
F.	3	6.3	312	306	312	324	318	314
M.	3	8	294	276	300	306	324	300
Group average.....		7.6						301
	7	10	360	344	314	321	315	331
	7	10	309	315	312	308	304	310
	7	10	308	285	300	288	279	292
	7	10	320	322	324	315	306	317
	7	10	316	292	303	291	288	298
Group average....		10						309
F.	20	20	417	426	411	420	360	407
M.	20	18	408	405	411	438	401	413
F.	20	19	548	522	534	504	572	545
M.	20	19	444	438	450	450	462	449
F.	20	19	576	636	600	630	678	624
Group average....		19						488

in most of the studies five only were made on each animal. Usually the rates were determined singly on the rats taken at random but in a few cases in which the conditions were particularly satisfactory several determinations up to the full five were made at once.

The further studies under contemplation involve the use of adult rats, hence in this work for the most part such only were used. As a matter of curiosity, however, and to demonstrate the wide applicability of the method, five determinations were made on each of three groups of five rats the ages of which were 3 days, 7 days, and 20 days, respectively. In the 3-day series the rats were taken from the nest and used at once

TABLE 4

Effect of restraint on pulse rate

Pulse rate per minute

RAT NUMBER	BASAL	HELD					AVERAGE	FETTERED					AVERAGE
8	280	558	468	525	519	528	520	720	696	705	699	699	720
9	262	468	513	528	453	495	492	585	642	606	591	543	593
11	280	382	468	528	486	498	472	612	552	546	594	630	587
19	301	504	483	569	474	468	500	648	612	672	654	666	650
23	317	528	528	666	666	654	608	618	678	684	684	684	670
25	290	522	540	552	588	570	554	612	684	636	636	642	622
29	320	528	540	636	624	540	574	684	696	666	678	663	677
33	295	600	534	537	534	404	522	666	660	642	624	618	642
39	297	598	513	540	537	525	543	662	624	624	624	645	637
41	290	492	519	480	549	558	520	708	624	704	615	618	654
43	284	525	484	444	534	558	509	672	666	594	612	633	635
68	305	600	627	656	642	636	632	750	768	750	792	750	762
70	305	546	588	636	678	666	623	702	690	666	666	618	668
72	310	612	636	654	630	576	622	760	672	757	710	727	725
74	315	612	618	612	618	576	607	702	744	720	696	672	707
82	335	360	356	384	402	360	374	618	690	672	660	654	659
106	344	558	522	576	618		568	660	684	588	678	684	659
107	316	588	576	570	600	582	581	594	576	612	612	606	600
112	340	426	432	462	480		450	606	630	636	624	624	624
114	319	492	480	480	492	504	490	574	630	636	648	660	630
Average..	305						538						656

without consideration of their poikilothermoid character. In the 7-day series they were kept at 35°C. for about an hour before the pulse rate was taken. The results are shown in table 3. Little difficulty was experienced in obtaining sharp and easily audible heart sounds even in the smallest animals. The point at which the sounds were best heard varied somewhat with the position of the animal but in general the spot giving the best results corresponded to the "punctum maximum" of the human subject.

In case of 20 animals the effect of excitement on the pulse rate was studied. After determining the approximately basal rate the animals were

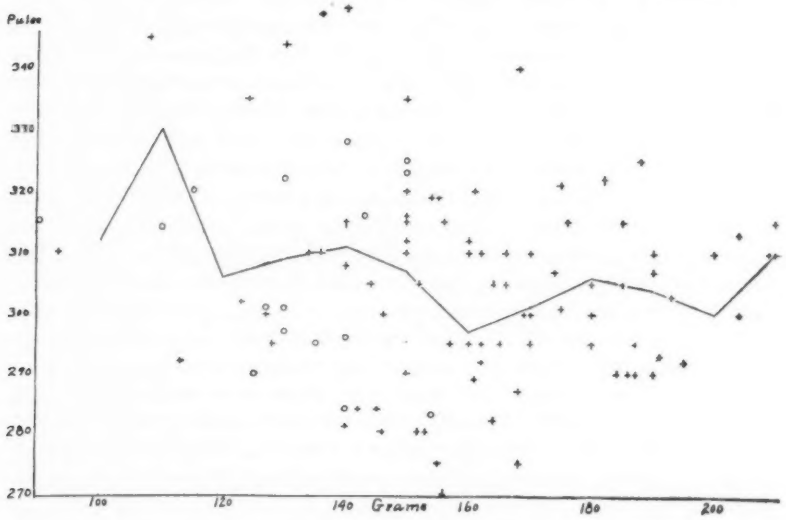

Fig. 2. Chart showing the pulse rate of sixteen female (o) and eighty-four male (+) rats, varying in weight from 92 to 210 grams. The line shows the average of each group of rats by successive increments of 10 grams weight. The plotted point in each case represents the average weight of the group up to that point.

held by an assistant comfortably and with as little constraint as was consistent with the necessary auscultation. The animals were then tied supine on an animal board, a procedure to which they manifested marked disinclination, and rate again determined. The results are recorded in table 4.

RESULTS. The results of the main study are set forth graphically in figure 2. The series included 84 males ranging in weight from 92 to 210 grams and 16 females varying from 94 to 165 grams, making 100 animals in all. The rate plotted for each animal is based on 10 to 40 individual counts through periods of 20 to 40 seconds each. The distribution of the

females as to weight being fairly uniform, and the number of females being relatively small, the rates of both sexes are plotted together. The average rate for the females was 307−; of the males 305− and of the total series of 100 animals was 305+ beats per minute with a mean deviation of ±19. The average rate for each group within 10 gram weight limits but irrespective of sex is plotted as the "average line" in the graph. Considering these data alone, there appears roughly the expected decline in rate with increase of weight. It is obvious, however, that with so much scattering a much larger series would be required to fix an absolute average that would be statistically valid.

There are two causes of deviation from the true average that must be borne in mind in interpreting the graph. The first is the error of the method. Both stop-watch checks and simultaneous recording of the rate by the auscultatory and by mechanical means show a maximum error for any five-count determinations of 5 per cent. It is probable that the actual error is somewhat less than this amount in that both checks were made, as previouly suggested, under less favorable conditions than were the pulse determinations. Since the human sense of hearing and neuromuscular activity were involved as one essential feature of the method the ability of the listener to synchronize sounds and signals comes into question. To one with a fairly well-developed sense of rhythm this difficulty, as a matter of fact, is not great. That the limit of ability to actuate the yoke key is about 900 signals per minute was demonstrated experimentally. Steady rhythms of 300 to 600 beats per minute were easily sustained, a feat obviously less difficult than that exacted of any reasonably proficient orchestral performer. Anyone who has listened to a full orchestra playing a quick movement will realize that even a hundred performers can simultaneously remain in rhythm within ranges of rate comparable to those involved in these experiments. The consistency of records obtained at random intervals on a given animal in a continuously steady state presents convincing objective evidence also of the accuracy of the method.

A more significant source of error is variation in the degree of alertness of the individual animal. In this series of studies a certain degree of variability was permitted in order to avoid the expenditure of an inordinate amount of time in waiting. In extreme cases the variation from this cause amounted to about 15 per cent of the average. Usually the variability was well under 5 per cent. Evidence was cited in a preceding paragraph that the rates recorded are some 8 per cent above true basal. Judging by the data set forth in table 2 and which are random selections, 2 per cent is a generous allowance for incidental variability.

Excluding the rats weighing less than 19 grams, the data indicate, as has been shown for numerous other animals, an inverse relationship between weight and pulse rate. In addition to the data of figure 2 those

in the third group of table 3 may be considered. In this group five animals averaging 19 grams in weight had an average pulse rate of 488. This rate is presumably somewhat above basal in that the animals were being held by an assistant rather than lying spontaneously relaxed. In each case, however, the observer's notes were to the effect that the animals were "quiet."

That quietude of the animals is a primary requisite is well shown in table 4. The twenty animals upon which the study was made gave an average rate of 305 as "basal." They were gently picked up by an assistant and placed on a desk before the auditor with care to avoid excitement or any unnecessary restraint. In general the rats accepted this treatment with little resistance. These manoeuvers, however, served to augment the rate to 538 beats. When the same animals were tied out upon a comfortable holder they resisted forcibly and continued to struggle more or less throughout the period of observation. In individual cases pulse rates above 750 were recorded. One rat averaged 762 beats for five consecutive determinations. The average rate for the twenty fettered animals was 656, well over twice the basal rate.

Discussion. Despite the suspicion attending the use of subjective methods the results of these experiments lead us to commend the method to those interested in the pharamco-dynamics of the heart. The errors incident to anesthesia or forcible restraint of the experimental animals greatly transcend those involved in this auscultatory method. Moreover, the expedition with which data can be obtained is a very attractive feature. In this connection it may be stated that by the use of a magnetic counter controlled directly by the auditor scores of observations can be made in a day, a fact that we have personally demonstrated.

SUMMARY

By means of a stethoscope and electric signaling system the pulse rate of 100 albino rats, varying in weight from 92 to 210 grams was determined. This rate varied from 270 to 350 beats, giving an average of 305. In younger animals the rates of three groups of 5 rats each averaging 7.6, 10 and 19 grams in weight were respectively 301, 309 and 488 per minute. In case of twenty animals comfortably held by an assistant the basal rate was elevated from 305 to 538 beats. When the same rats were forcibly fettered on an animal board the rate was further increased to 656. The method employed is recommended to those interested in pharmaco-dynamic studies on the heart.

EXPERIMENTAL CRETINISM

I. A Rachitic-like Disturbance in Extreme Hypothyroidism[1]

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Findly (1908) and Mellanby (1919) clearly demonstrated that rickets occurred in young animals fed on a diet containing an insufficient amount of certain substances now known as the "antirachitic vitamine." As a result of this, the British Medical Research Committee announced that rickets is a deficiency disease due to a lack in the diet of an anti-rachitic factor. For literature up to 1923, covering the etiology of rickets with special reference to dietary deficiencies, the curative values of cod liver oil and of ultraviolet radiation and the importance of the salt composition of the diet in this disease, the reader is referred to the excellent reviews of Korenchevsky (1921) and of Park (1923).

Inasmuch as the present report deals entirely with disturbances in the growth and calcification of the bones of young rabbits, due to causes other than dietary factors, brief mention should be made of the literature on the etiology of rickets dealing with the problem from other aspects than dietary deficiencies. Park and McClure (1919) reviewed the literature on the effects of thymus extirpation on young animals and report results of their own findings on 24 out of 75 thymectomized puppies; 19 control dogs from four different litters were used. These authors present photomicrographs of the long bones and ribs of their experimental animals and conclude that no signs of rickets appear at the junction of the cartilage and shaft, and that extirpation of the thymus does not influence growth or development but the possibility of a retardation in development or a delayed closure in the epiphysis cannot be absolutely excluded. Park and McClure did not make chemical analyses of the calcium and phosphorus content of the blood.

Relative to the influence of the pancreas on the production of rickets, Park and McClure (1919) state that "Pawlow observed pathological conditions of the skeleton in the nature of osteomalacia after various kinds of operations in the pancreas region and after the production of intestinal

[1] This work has, in part, been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

fistulae, and Fisher and Looser, after operations on the bile ducts." Betke (1915) removed both carotid bodies and describes changes in growth and the production of rickets resulting from his experiments. Korenchevsky (1922) reviews the literature on the influence of parathyroidectomy on the skeleton of animals normally nourished and on rickets and osteomalacia produced by deficient diets. Korenchevsky shows that the histological pictures through the costochondral junctions of his three rats parathyroidectomized and receiving a normal diet did not differ essentially from those of the control animals. One of these showed a very slight amount of increase in the endothelial osteoid tissue and a slight osteoporosis and narrowing of the proliferating cartilage which was completely impregnated with calcium.

An undersized condition has been described in children and young adults, which is accompanied by a severe form of chronic interstitial nephritis or developmental defects of the kidneys. These subjects had rachitic deformities. This syndrome has been called renal dwarfism.

Hofmeister (1894), (1896), (1897–1898) successfully thyroidectomized young rabbits and noticed a retardation in the growth of the long bones as determined by actual measurements of the tibia and ulna, and a delay in the disappearance of the epiphysis. Hofmeister gave the name of chondrodystrophia thyreoprevia to this condition and declared it to be identical to chondrodystrophia foetalis. He also states that histological section through the epiphysis presents the same picture as the so-called fetal rickets. This author also cites a clinical case of Kocher dating back to 1883, where post-mortem findings reveal a disturbance in the development of the epiphysis of a girl aged 20 and thyroidectomized at 11 years.

If we except the experiments of Park and McClure on the thymus and of Korenchevsky on the parathyroids we find that in most instances no consideration has been taken of dietary or hygienic influences on the production of rickets, where this condition is said to occur as the result of some glandular or operative disturbance. In most instances the reports were published before the importance of the diet and environmental conditions had been experimentally demonstrated. With these factors well known, the present investigation was planned to determine whether or not thyroidectomy in the very young rabbit living on a diet adequate in every respect for normal rabbits, would result in disturbances in the developing skeleton other than a diminution in growth.

EXPERIMENTAL. Young rabbits between 2 and 3 weeks after birth were thyroidectomized according to the method outlined by Hofmeister (1894) and described in detail by Tatum (1913) and by Basinger (1916); one or two rabbits from each litter were not thyroidectomized; these served as controls and received exactly the same dietary and were subjected to the same environmental conditions as their thyroidectomized litter mates.

In most instances control and cretin occupied the same cage. The dietary consisted of unrolled oats, an abundance of high grade alfalfa leaf, carrots, and a small amount of yellow corn, with water *ad libitum*. The rabbits were housed in a moderately well-lighted laboratory, but (with some exceptions which will be explained later) were not exposed to the unfiltered rays of the sun.

The evidence we present was obtained entirely from the operated animals which actually suffered from marked thyroid deficiency during the

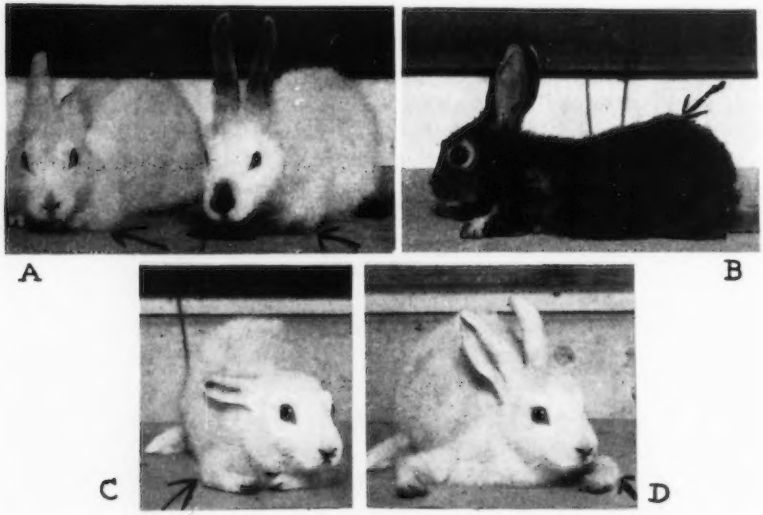

Fig. 1. *A*, *B*, *C* and *D* are photographs of different litters of rabbits, thyroidectomized between 2 and 3 weeks after birth. The arrows point out the rachitic deformities which occur in these animals living on a nutritious diet containing an abundance of antirachitic substances. The housing conditions of these rabbits were such that they were not exposed to the unfiltered rays of the sun.

growing period as determined from a comparison of the growth curves of the operated animals and the normal litter mates. An apparently complete thyroidectomy very frequently results in little or no thyroid deficiency due, presumably, to accessory thyroids or to hyperplasia of a few thyroid cells which remained after the operation. The growth curve of such animals during the first three months after operation approximates the curve of a normal rabbit. Such animals were discarded from the experiment. Occasionally an animal shows marked thyroid deficiency during the first three months after operation but later grows fairly well, due

perhaps to more effective functioning of accessory glands or hypertrophied thyroid tissue. These animals also show marked disturbance in bone development which results in permanent deformities (see fig. 1, *D*).

From the spring of 1923 up to the present time (June 6, 1927) 404 rabbits out of a much larger number of thyroidectomized animals have shown marked thyroid deficiency and were chosen for the study of rickets in experimental cretinism.

The *gross findings* of these animals which indicate a disturbance in the development of the skeleton consist chiefly in marked dwarfing of growth, flatfooted posture, bowing of the forelegs, lateral displacement of the clavicles, bulging of the occipital and parietal bones and marked kyphosis. In addition to this the animals early (4 to 8 weeks after operation) developed a pronounced potbelly and later (3 to 4 months after operation) the coat became shaggy and the skin covered with scales as described for cretin condition in rabbits (Hofmeister, Tatum, Bassinger).

Roentgenograms of the fore legs of a cretin five weeks after operation, and its control litter mate are shown in figure 3, *A* and *B*. In the cretin the following deviations from the normal are seen: The distal ends of the radius and ulna clearly show the increased width of the epiphyses, the translucent metaphysis, the narrow zone of calcification at the epiphyseal end of the bones and the lessened density of the shaft of the long bones. Figure 3, *C* and *D*, shows roentgenograms of cretin and control 5 months later. Here the distinct bending of the long bones of the cretins' front legs is apparent, also the still open epiphysis.

Histological examinations of longitudinal sections through the epiphyseal ends of the long bones (fig. 2, *B*) give evidence of excessive multiplication of cartilage cells as denoted by the enlarged zone of proliferation. Near the metaphysis the cells are larger and the columnar arrangement less clearly defined. This proliferating zone is incompletely or entirely devoid of calcium. An abundance of osteoid tissue separated by wide medullary spaces, and a diminution or absence of normal bone trabeculae are apparent in the metaphysis.

Blood examination. Chemical analysis of the blood (table 1) shows that the acid soluble phosphorus is markedly below normal, whereas the calcium content is normal. Occasionally the calcium is very slightly below that of the normal litter mate but usually falling within the lower limits of normal variation. Preliminary reports have been made on the marked anemia and the increase in cholesterol of the blood which is found in this condition (Kunde, 1926).

Discussion of results. Our experiments demonstrate that when rabbits are thyroidectomized between 2 and 3 weeks after birth and suffer marked thyroid deficiency as indicated by the growth curve, a pathological condition develops which corresponds in all fundamental respects to rickets

in human beings. This is not due to a deficiency in the anti-rachitic vitamines of the dietary inasmuch as the unoperated litter mate living in the same cage and sharing the dietary of the operated animal shows no disturbance in the skeletal development. Moreover, the dietary consists of an abundance of high grade alfalfa leaf, unrolled oats, a small amount of yellow corn, carrots and water *ad libitum*. McCollum et al. (1917) showed that a dietary consisting of 40 per cent alfalfa leaf and 60 per cent rolled oats was sufficient for normal growth and the rearing of young, and later Steenbock and Gross (1920) demonstrated that where 15 per cent of the rations of the white rat consisted of alfalfa, the requirements for the fat and water soluble vitamines were satisfied, i.e., normal growth and reproduction followed. These same authors (1919) also found that where 15 per cent of the dietary consisted of carrots, normal growth and the rearing of young occurred. In our rabbits the disturbance of the bone development is not due to a deficiency in the dietary but to an inability to properly utilize the essential substances present in the food in great abundance. The marked lowering of the metabolic rate in very young growing rabbits due to thyroidectomy soon develops into a general metabolic disturbance with a syndrone of signs comparable to a severe disturbance in nutrition brought about by feeding diets insufficient in certain essential factors.

The blood is also included in this metabolic disturbance. After several weeks of induced cretinism a severe anemia develops (Kunde, 1926). The details of this will be reported later. It is quite possible that the rickets which develop in the animals is secondary to this anemia. This rachitic condition readily responds to thyroid medication, as can be seen by increased calcium deposition in the zone of provisional calcification of the epiphyses after desiccated thyroid has been given to these rabbits daily for a short period of time (fig. 2, *C*). This supports the theory of Lanz (1894) who suggested the use of thyroid in rickets, but does not agree with

Fig. 2. Photomicrographs of longitudinal sections through the epiphysis of the distal end of the femur of 3 littermates 13 weeks old, living on the same dietary and under identical conditions of light. *A* = normal unoperated rabbit; note the narrow, uniformly arranged columns of the epiphysial disk of cartilage which is completely calcified, the proliferating layer being only 1 to 3 cells deep and the beginning of the diaphysis containing the normal amount of bony trabecula. *B* = the littermate thyroidectomized between 2 and 3 weeks after birth; note the marked increase in the width of the epiphysial disk of cartilage, the absence of calcium in the wide proliferating zone of cartilage and the irregular arrangement of the uncalcified cartilage. The metaphysis contains an overproduction of osteoid tissue. *C* = Thyroidectomized rabbit 4 weeks after daily doses of thyroid had been given; note the complete calcification of the proliferating zone of cartilage and the calcium deposits in the metaphysis. Magnification × 52 (hematoxylin and eosin). *bt.* = bony trabecula; *e. car.* = epiphysial disk of cartilage; *e. cav.* = epiphysial cavity; *ost.* = osteoid tissue.

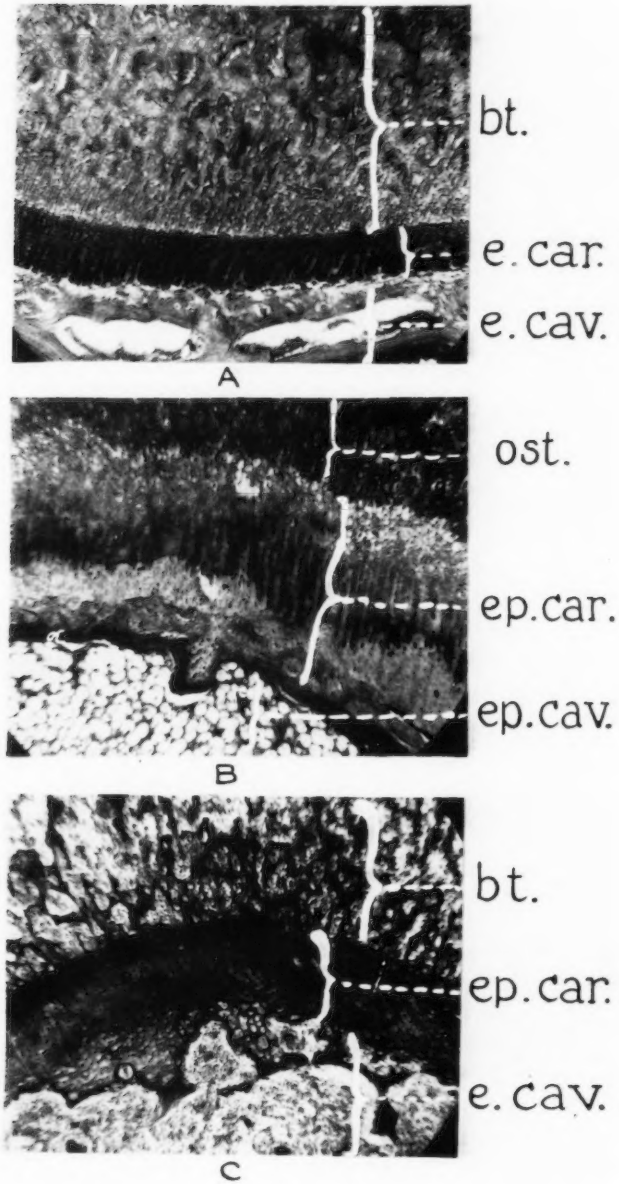

Fig. 2

the results of Knoeppelmacher (1895) and of Heubner (1896) who report no effect of thyroid medication. Clinically, it has been shown that many children with marked secondary anemia develop rickets. The deformities in this type of rickets differ slightly from those ordinarily seen, in that

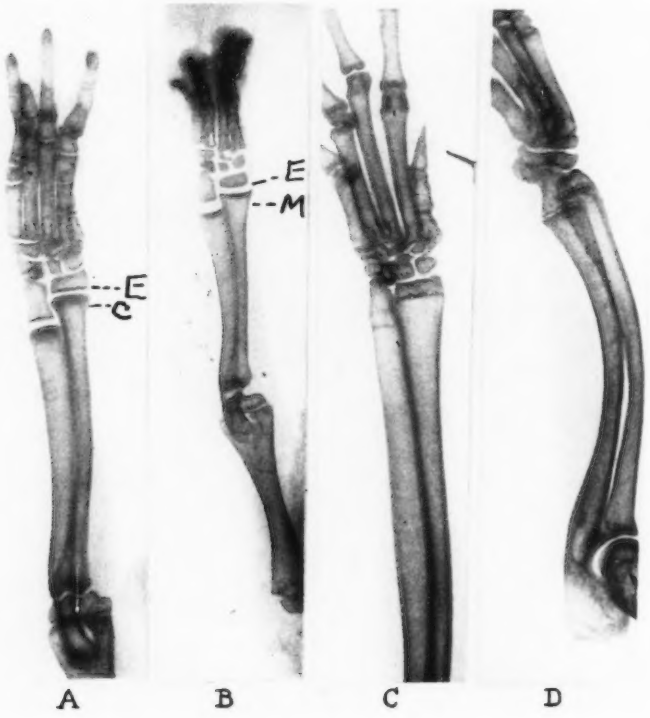

Fig. 3. *A* and *B* are roentgenograms of littermates. *A* = normal animal; *B* = cretin 7 weeks old thyroidectomized 2 weeks after birth. Note the difference in the width of the epiphysis, *E*, of the normal and cretin animals, also the calcium deposits, *c*, at the beginning of the normal shaft, whereas in the cretin the metaphysis, *M*, shows very little calcium. *C* and *D* are roentgenograms taken 5 months later. Note the bending of the radius and ulna of the cretin, *D*, and the straight lines of the normal animal, *C*.

the enlargements of the ends of the extremities and at the costochondral junctions are not so marked as in rickets due to dietary deficiencies. These moderate enlargements describe the condition in our experimental animals.

The low acid soluble phosphorus in the blood, and the normal or very

slightly below normal calcium content of the blood of our cretin rabbits corresponds to the reports of Kramer and Howland (1921); these authors made chemical analyses of the concentration of the calcium, phosphorus and magnesium in the sera of rachitic children and found that, excluding frank tetany, the calcium content of 12 cases out of 25 fell within normal limits and with the remaining 13 cases the calcium decrease was not significant. But the acid soluble phosphorus of the sera was markedly below normal. Our experiments show that the type of rickets which occurs in cretin rabbits living on an adequate diet is characterized by a low acid soluble phosphorus of the serum. Shipley et al. (1922) showed that this type of rickets (normal calcium and low phosphorus in the serum) could be produced in rats by diminishing the phosphorus and supplying the optimal amount or an excess of calcium in the diet, provided the diets contained an insufficient amount of the substance found in cod liver oil, or if the rats were deprived of certain active light rays.

TABLE 1

Showing variations in the blood calcium and in the acid soluble phosphorus of the serum of normal and of cretin rabbits

CONDITION OF ANIMAL	NUMBER OF ANIMALS USED	PHOSPHORUS ACID SOLUBLE PER 100 CC. SERUM			CALCIUM PER 100 CC. SERUM		
		Highest	Lowest	Average	Highest	Lowest	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Normal.....	4	5.6	4.2	4.6	14.9	13.5	14.2
Cretin.....	9	3.7	2.9	3.4	15.	13.08	13.7

Our rabbits were housed in a moderately well lighted laboratory but not exposed to the unfiltered rays of the sun. Under these conditions no unoperated rabbit ever showed signs of rickets, showing that under normal conditions this environmental factor did not contribute to the production of the disease. Recent experiments seem to indicate that the type of rickets which occurs in cretin rabbits (not of dietary origin and not responding to a normal diet) is improved by certain active light rays. The evidence in this respect is not sufficient at present to justify definite conclusions.

The investigations of disturbances in skeletal development were all made during the phase when the rabbits were still growing though growth was markedly delayed. After growth ceases in certain absolute cretins, which rarely live more than 10 to 12 weeks after operation, the epiphysis becomes narrow and the picture of active rickets can no longer be clearly demonstrated.

SUMMARY

Cretin rabbits (thyroidectomized between 2 and 3 weeks after birth) develop a condition of disturbance in skeletal development which fundamentally simulates clinical rickets. This is not due to a dietary deficiency. This rickets-like condition is accompanied by severe anemia.

This ricket-like condition is characterized by a normal or slightly below normal concentration of the blood calcium and a low acid soluble phosphorus of the serum.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD; ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

IV. Ammonia Nitrogen

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Like that of the other constituents which have been reported in previous articles of the series, it was our intention to include the ammonia determination of the blood but when it was discovered that reliable data could be obtained with difficulty the project was abandoned. Consequently, we are herewith offering data on only the urine and the sweat.

The determinations were made by the colorimeter method of Folin and Bell. The work, like that reported in the previous articles of the series, was performed in two parts, the first of which was done during the summer of 1925 by Mr. Finkle while the second part was done by Mr. Katsuki during the summer of 1926.

Urine. The amount of ammonia nitrogen in the urine showed quite a marked fluctuation from the minimum of 0.17 to maximum of 3.26 mgm. However, it must be noted that these extremes represent the exception rather than the rule. The analyses were started immediately after collection. There were 79 experiments in all, of which 39 were done in 1925 and 40 in 1926. As reported in previous articles, the first sample of urine was taken shortly before sweating and was used as a control, while the second sample was taken about 20 minutes after retiring from the sweat cabinet. The third and fourth samples were collected at hourly intervals respectively. However, the fourth sample was omitted on several occasions, and in a few instances the first or second sample was unavoidably omitted.

In comparing the urine after sweating with that of the control, it will be noted in table 1 there was an increase in the ammonia nitrogen in a trifle over 80 per cent of the cases. A like comparison in table 2 shows an increase in 70 per cent of the cases or both taken together, will show an increase in 75 per cent of the cases.

In consulting chart 1 there will be observed a 50 per cent correlation when the ammonia nitrogen is plotted against the urea nitrogen of the second samples of urine which are collected immediately after sweating.

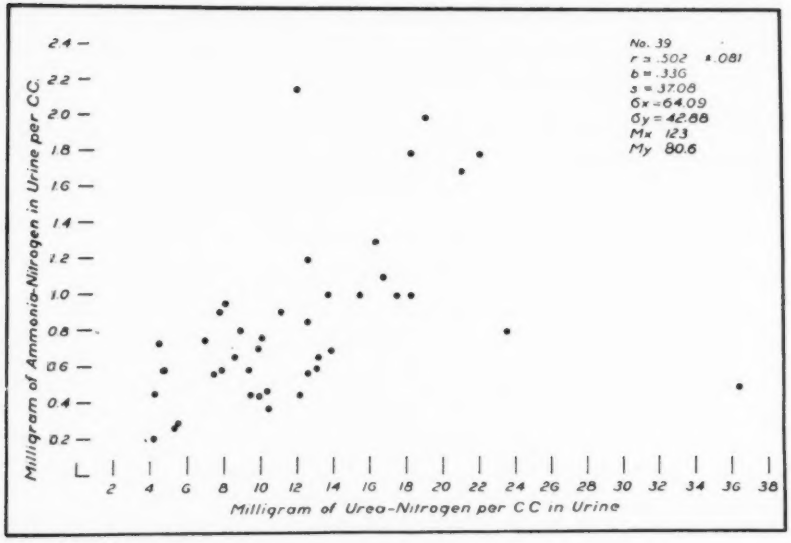

Chart 1

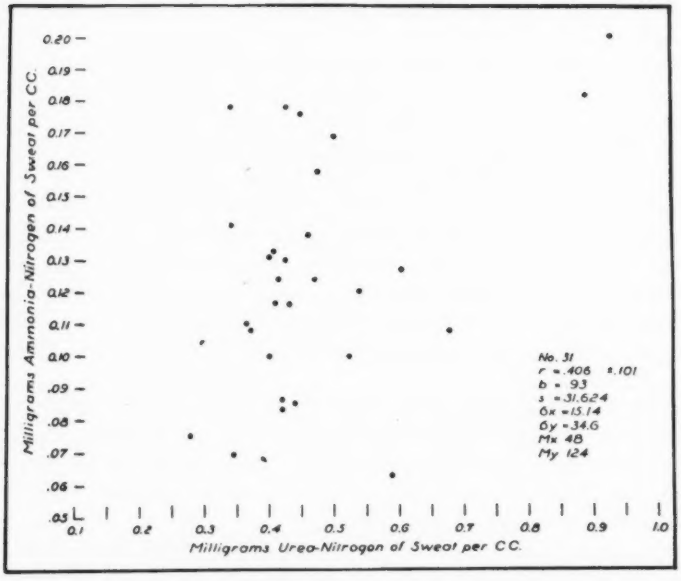

Chart 2

TABLE 1

Ammonia nitrogen in urine and sweat in milligrams per cubic centimeter (1925)

SUBJECT	URINE 1	URINE 2	URINE 3	URINE 4	SWEAT
F. C.	0.54	0.48	0.30		0.16
F. C.	0.23	0.83	0.92		0.09
F. C.	0.41	0.77	0.49		0.06
F. C.	0.64	0.85	0.57		0.10
F. C.	0.31	1.01	1.05		0.05
F. C.	0.54	1.10	0.72		0.05
F. C.	1.10	3.25	0.95		0.06
F. C.	0.39	0.72	0.64		0.09
F. C.	0.22	0.52	0.26		0.09
F. C.	0.52	1.08	0.44		0.11
F. C.	0.26	0.51	0.38	0.21	0.10
F. C.	0.32		0.83		0.05
F. C.	0.32	0.51	0.22		0.05
F. C.	0.72	1.15	0.90		0.17
F. C.	0.18	0.37	0.33		0.07
F. C.	0.78	0.72	0.62	0.79	0.17
F. C.	0.78	0.20	1.19	0.74	0.06
F. C.	0.31	0.47	0.88	0.61	0.35
F. C.	0.73	0.84	0.73		0.13
F. C.	0.52	0.54			0.04
F. C.	0.51	0.85	0.73		0.06
F. C.	0.35		0.59		0.04
F. C.	1.37	1.43			0.09
F. C.	0.86	0.94			0.20
F. C.	0.75	0.48	0.61		0.10
F. C.	1.02	1.19	0.34	0.14	0.05
F. C.	2.60	2.10			0.07
F. C.	0.28	0.41	0.26	0.39	0.06
F. C.	0.64	1.23	0.67	0.65	0.08
F. C.	0.72	0.58	0.73	0.73	0.04
C. H.	0.47	0.89	0.99	0.64	0.07
C. H.	0.50	0.97	1.77	0.99	0.10
C. H.	1.19	2.04	1.90		0.07
C. H.	0.85	1.32	1.21	0.63	0.07
C. H.	0.65	1.12	1.16	1.08	0.09
C. O.	0.60	0.39	0.50		0.06
C. O.	0.53	0.68	1.08		0.11
C. O.					0.05
R. T.	0.38	0.83	1.21	1.24	0.15

TABLE 2

Ammonia nitrogen in urine and sweat in milligrams per cubic centimeter (1926)

SUBJECT	URINE 1	URINE 2	URINE 3	URINE 4	SWEAT
H. I.	0.40	0.59	0.34	0.25	0.07
F. C.	0.66	0.49	0.37	0.55	0.25
C. H.	0.55	0.46	0.81	0.95	0.11
F. C.	0.94	0.85	0.46	0.39	0.12
W. G.	0.66	0.73	0.79		
S. S.	0.42	0.66	0.55	0.97	
W. G.	0.87	1.00	0.96		0.14
J. A.	0.37	0.43	0.51	0.52	0.06
F. C.	0.49	0.58	0.47	0.65	0.10
L. M.	0.41	0.44	0.39	0.43	
F. C.	0.29	0.44	0.38	0.37	0.18
J. A.	0.15	0.28	0.23	0.26	0.07
F. G.	0.33	0.58	0.49	0.52	0.10
F. C.	0.81	0.56	0.72	0.57	0.18
G. M.	0.34	0.20	0.28	0.72	0.10
G. B.	0.94	0.37	0.56	0.95	0.12
F. C.	0.82	0.69	0.68	0.65	0.09
J. A.	0.47	0.58	0.62	0.50	0.18
I. H.	0.79	1.00	0.99	0.57	0.12
W. K.	0.28	0.45	0.39	0.42	0.13
I. R.	1.05	1.00	1.33	1.32	0.12
R. B.	1.18	1.25	2.67		0.13
C. H.		1.13	0.87		
F. C.		0.70	0.68		0.16
J. A.	0.30	2.60	0.47		0.12
G. M.	1.41	0.76	0.55		
W. K.	0.89	0.90	1.39		0.24
F. C.	1.26	1.65	1.41		0.17
I. R.	0.49	0.75	0.61		0.16
W. K.	0.86	1.05	1.16		0.18
C. H.	0.77	0.80	0.79		
J. A.	0.44	0.79	1.25		0.12
I. R.	0.44	0.56	0.56		0.21
F. C.	1.82	1.82	1.53		0.13
O. H.	0.68	0.90	1.17		0.14
J. A.	1.10	1.80	1.67		0.13
F. C.	1.66	2.00	2.20		0.11
W. K.	0.63	1.66	1.33		0.07
C. H.	1.11	0.17	0.11		0.11
O. H.	0.66	0.95	1.23		0.08

Sweat. In 73 determinations the sweat yielded a minimum of 0.05 to a maximum of 0.35 mgm. per cubic centimeter. The latter figure is, however, unusually high. In chart 2 where the ammonia nitrogen is plotted with the urea nitrogen of the sweat, there will be observed a correlation of 41 per cent. When the ammonia of the sweat is plotted with the ammonia of the urine, no correlation is to be observed.

SUMMARY

1. The amount of ammonia nitrogen in milligrams per cubic centimeter is reported in the urine and the sweat.

2. The increase in the ammonia in the urine as a result of sweating is indicated.

3. The extent of correlation of the ammonia nitrogen and the urea nitrogen in the sweat and urine is noted.

THE INTERPRETATION OF THE ACTION POTENTIAL IN CUTANEOUS AND MUSCLE NERVES

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Due to the fact, brought out in a previous paper (1927), that the relation of fiber diameter to conduction rate is one of direct proportionality, the configuration of the action potential started by local stimulation of all of the fibers of a nerve depends upon the fiber-size pattern of the nerve and the distance of conduction. The action potential in the sciatic nerve of the frog (*Rana pipiens* and *Rana catesbiana*) on this account consists, provided the distance of conduction be sufficiently long, of three distinct waves corresponding with three more or less distinct groups of fibers in respect to diameter. For the sake of convenience the waves in this action potential were designated *alpha*, *beta* and *gamma* in the order of their appearance in the compound potential. At times an indistinct fourth, or *delta*, wave is seen. Waves are also seen in the action potential of the saphenous nerve of the dog, and these likewise were given the noncommittal designations of *alpha*, *beta*, etc., in the order of their conduction rates. Recent observations, however, have shown that the first wave in the saphenous nerve is not homologous with the first wave in the sciatic, but rather with the second. The observations pointing to this conclusion and the light they throw on the question of a possible relation of the fiber grouping to function form the subject of this brief communication.

Evidence indicating that the action potentials of the sciatic and saphenous nerves might not be comparable wave for wave was obtained in an experiment which showed that the rate of conduction is faster in a muscle branch of the femoral nerve than it is in the saphenous branch, which, as is well known, contains no voluntary motor fibers. The preparation employed in this experiment (of which several have since been performed) consisted of the dog's femoral nerve with the saphenous branch and one of the branches supplying muscles of the thigh attached, both cut to exactly the same length. The preparation was mounted in our moist chamber at body temperature and arrangements were made to stimulate the femoral nerve with induction shocks while leading monophasically, and in succession, from a point on each of the two branches, both equidistant

from the site of stimulation, through the amplifier into the cathode ray oscillograph according to the technique described elsewhere (1922) (1924).

The records obtained in such experiments show clearly (fig. 1, *A* and *B*) that the front of the action potential in the muscle nerve arrives at its lead

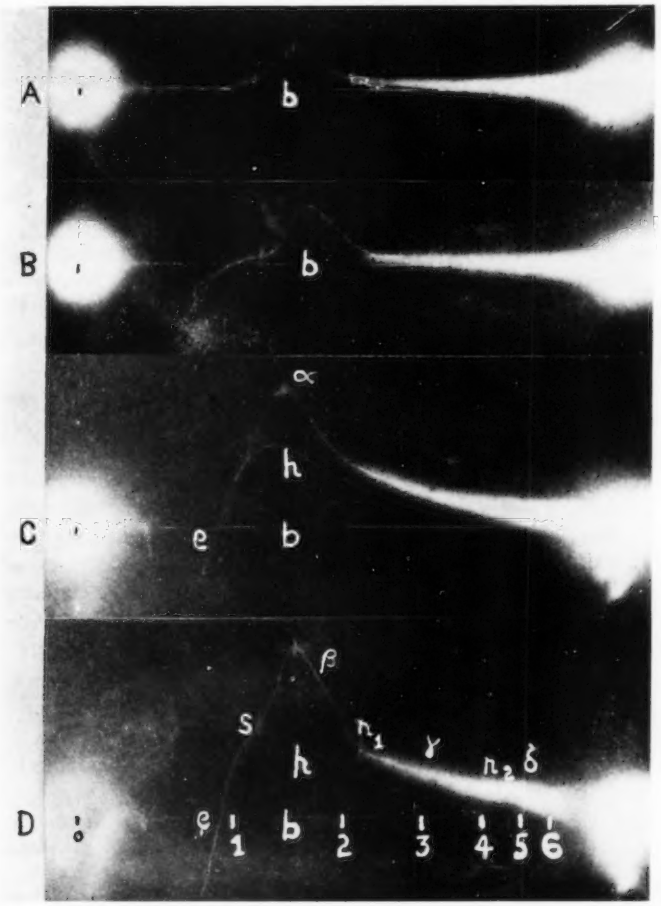

Fig. 1. Records from a femoral-muscle-saphenous nerve preparation of the dog. Femoral stimulated; lead from muscle nerve in *A* and *C* and from saphenous in *B* and *D*. The same weak stimulus in *A* and *B*, and strong stimulus in *C* and *D*. Relative heights inconsequential. Conducting distance 7 cm. *e* = exposed escape; *h* = covered escape (assumed form). *S* = start of action potential. *O* = base line. A faint pencil line has been drawn through the deflection in *B*, *C* and *D*. *X* = 81 mm., 3000$^{\sigma}$, T = 37°. The time for all is indicated in σ in *D*. Natural size.

a bit earlier than the front of the action potential in the saphenous. The calculated conduction rates of the wave fronts in the case of figure 1 are 92 and 84 m.p.s. in muscle and saphenous nerves, respectively. Differences in conduction rate of this order of magnitude are invariably obtained, though the absolute conduction rates may vary considerably depending upon circumstances. The rates in this particular case were somewhat above those usually obtained.

The records, *A* and *B*, figure 1, reproduced to show the differences in conduction rate, were elicited by a relatively weak stimulus. In order to obtain from this preparation complete action potentials it is necessary, owing, in part, to the low irritability of the slowest conducting fibers and, in part, to the thickness of the femoral trunk, to use very strong shocks, and these have the drawback of producing prominent escapes. When long nerves are available this matters but little for the escape then dies away before the action potential records; but in these experiments, in order to obtain exactly comparable records, it was necessary that the two branches be equal in length. The length of the preparation was determined, therefore, by the shorter of the two branches, and even though the dogs were the largest obtainable, the distance of conduction never exceeded 8 cm.

The best pair of action potentials elicited from the femoral-muscle-saphenous nerve preparation by sufficiently strong stimulation to bring out all of the main waves are reproduced as *C* and *D* in figure 1. The action potentials here are recorded on a prominent escape, *e*, the form of which under the action potential can only be surmised. Nevertheless, we have drawn into each record in exactly the same form, amplitude and position, a curve, *h*, which, experience indicates, probably comes very close to reproducing the unmodified form of the escape. It is this curve, presumably, that forms the base line of these two action potentials. The undeflected course of the spot of light (that is to say, the base line of the record as a whole) is indicated by the faint dotted horizontal line, *b*.

In these records may be seen all of the significant differences between the action potentials of these two branches. The faster propagation of the front of the action potential in the muscle nerve, *C*, is clearly indicated by its earlier position in the escape. In the sector marked γ record *D* is less concave upwards and further removed from the curve *h* than is *C*. And, finally, record *D* has at δ a low but distinct wave which does not appear in *C*. To elicit this last wave it was necessary to use the very strong stimuli to which reference has just been made.

In another place (1927) it has been shown that one can obtain, by means of a reconstruction based upon the fiber-size pattern of a nerve, the approximate form of the action potential at any distance from the site of stimulation. For this purpose it is necessary in any particular case to

ascertain only the diameters of a relatively large number of the fibers and the conduction rate in one (the largest, that is, the most quickly conducting) of the fibers of known diameter. The construction utilizes the assumptions, which probably are not far from the truth, *1*, that the time constants of all of the axon action potentials of a nerve are alike, the rising and falling phases in warm-blooded nerve under normal conditions being 0.2 and 0.4σ, respectively, in frog's nerve 0.3 and 0.6σ; *2*, that the rate of propagation of the axon action potential varies directly as the fiber diameter, and *3*, that the potentials of all the fibers are alike in amplitude but affect the recording instrument in proportion to their cross areas, that is to say, inversely as their electrical resistances.

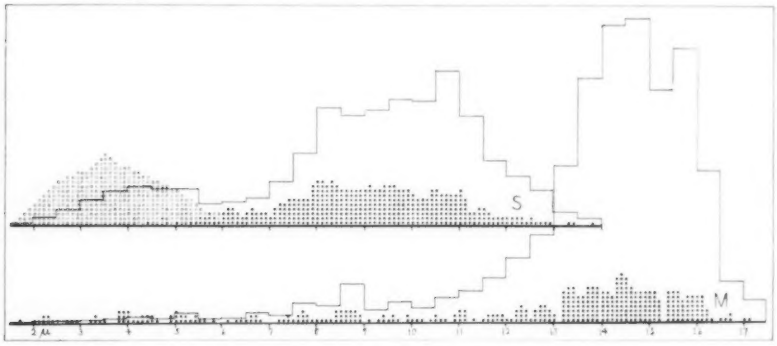

Fig. 2. Fiber-size patterns of two branches of the femoral nerve: M = a muscle branch (372 fibers measured); S = saphenous nerve (390 fibers measured (dots) and 330 estimated (circles)), about 30 per cent of the area of each section having been measured. Solid lines: total area of underlying fibers, the ordinates being on an arbitrary scale but the same in M and S. Abscissae: fiber diameter in μ.

Figure 2 shows the fiber-size patterns of a muscle branch, M, and of the saphenous branch, S, of a femoral nerve of the dog made in the manner previously described (1927). In the case of the saphenous nerve the diameters of the smallest group of myelinated fibers, from about 6 to 5μ down, were not measured because they were not clearly enough defined to permit of accurate measurement; instead they were merely counted and arbitrarily set in the graph in the form of a symmetrical mound between the smallest fibers actually measured, 5 to 6μ, and the roughly estimated size of the smallest fibers counted, about 2μ. Unmyelinated fibers could not be recognized as such, though masses of them undoubtedly were present.

In figure 3 are shown the action potentials of the muscle nerve, M, and of the saphenous nerve, S, reconstructed according to the scheme above indicated. The conduction rate in the fastest fiber, in other words, the

largest fiber, which, in the part of the nerve examined had a diameter of 17μ, has been taken as 92 m.p.s., and the distance of conduction as 7 cm. The reconstructed action potentials, therefore, are comparable with those in figure 1.

The resemblance of the reconstructed to the actual action potentials is quite obvious. In the case of the muscle nerve both consist essentially of a single large wave which travels faster than the front of the saphenous wave. The reconstructed potential possibly gives indistinct evidence of a second wave beginning at about 1.0σ. Were there a comparable wave in the record it would be small beyond recognition; for in the reconstruction it rises above the level to which the curve would fall, were it without this slight elevation, only a very small fraction (1/30 at most)

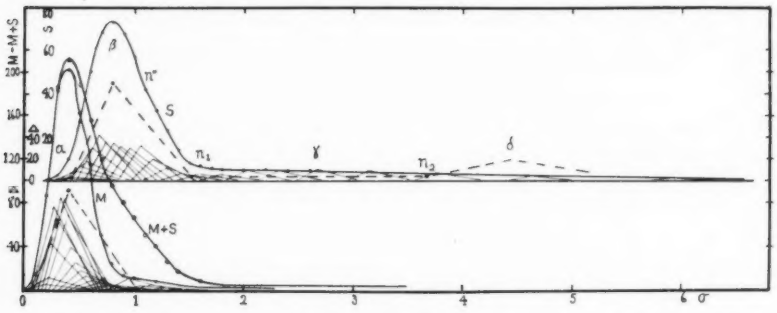

Fig. 3. Reconstruction (solid line curve) of action potentials in muscle branch, M, and saphenous branch, S, of a dog's femoral nerve at 7 cm. $M + S : S$ added to M. The points joined by the dotted lines represent accurately in time, but arbitrarily in amplitude, the corresponding points in C and D as the case may be, of figure 1, the main crest of the dotted curve being synchronous with the main crest of the reconstructed curve in each case.

of the height of the main wave. The long tail on the reconstructed curve, if present in the record, likewise would be low beyond recognition. The only real difference is in the duration of the rising phase of the main wave, which is longer by 0.1σ in the reconstruction than in the record.

It is convenient, at this point, to refer to certain factors which might have the effect of masking super-imposability of record upon reconstruction. In the first place, the start of the recorded action potential, on account of its gradualness, is the feature that is the most difficult to locate accurately; and this difficulty is increased when, as in case of records C and D, the escape extends under the action potential. On this account the times to maximum here employed are those obtained from records 1 and 2 where the difficulty due to the escape is practically absent. The crest of the main wave, on the other hand, is the feature that can be most accurately

located. Therefore, in superimposing the records upon the reconstructed action potentials in figure 3 the main crests have been made to coincide in point of time and the time points derived from the records have been laid off with respect to this crest. These points have been joined by the dotted lines. Another possible cause of failure of complete superimposability is the incompleteness of the analysis of the nerve, only about 30 per cent of the fibers in each of the branches having been measured. In view of the fact, however, that we have another reconstructed saphenous action potential, likewise based on a fiber-size pattern, that has a configuration almost identically the same as the one figured here, it seems unlikely that all of the discrepancy can be attributed to this source. In any event the differences seem insignificant and, for the reasons given, may be apparent rather than real.

In the case of the saphenous branch, the two curves differ from each other in one striking respect: the record exhibits three waves, the reconstruction but two. The third wave, labelled δ, is travelling at the rate of about 18 m.p.s. Its start coincides, in the reconstruction, with the group of fibers measuring 3.0 to 3.5μ in diameter, its crest with the 2.5 to 3.0μ group. The unmyelinated fibers of the nerve, which are not taken into account in the reconstruction, might very well produce an action potential wave answering this description. Furthermore, the absence of the δ wave in the muscle nerve and its presence in the saphenous is consistent with the fact, first established by Sherrington in 1894 and repeatedly confirmed since, that in skin nerves there are numerous unmyelinated fibers, whereas in nerves to skeletal muscles they take up only a very small part of the transverse section. Without doubt, therefore, it is the unmyelinated fibers that determine the third elevation in the action potential of the saphenous nerve. It follows from this that the unmyelinated fibers conduct at about the same rate as the myelinated fibers that measure from about 3.0μ in diameter down.[1] As in the case of the muscle nerve,the recorded time to maximum of the first wave is briefer (by 0.2σ, here) than the reconstructed time.

Another exemplar of a comparison of a reconstructed with a recorded action potential of the saphenous nerve will be included because of the clear manner in which it supports the view of a direct relationship between fiber diameter and conduction rate. It is obvious that the longer the

[1] The direct relationship between conduction rate and fiber-size was established through measurement of the over-all diameters of myelinated fibers. If the conduction rate in nerve fibers varies as the diameters of their axis cylinders, and if Donaldson and Hoke's (1905) contention is right, that the sheath comprises half the area of the whole fiber, it would follow that the most quickly conducting unmyelinated fibers that produce an impress on the recorded action potential measure, roughly, about 2μ in diameter. This does not seem to be unreasonable.

nerve the better separated will be the features that develop as a result of differences in axon conduction rates. Figure 4 is a record of the action potential in the saphenous nerve picked up at a point 15 cm. from the site of stimulation. The conduction rate was about 60 m.p.s. Figure 5 shows the reconstruction at 15 cm. derived from the fiber-size analysis of the saphenous nerve. The dotted lines join points whose positions, temporally, are the same as in the record; they consequently indicate diagrammatically the configuration of the recorded action potential in relation to the constructed action potential. The agreement wave for wave is not perfect, but the greatest discrepancy amounts to only 0.2σ.

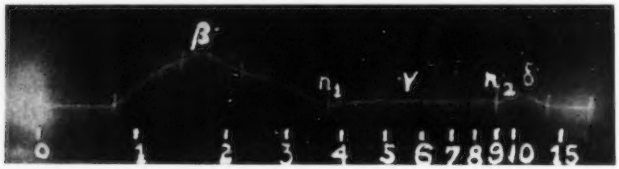

Fig. 4. Record from the saphenous nerve of the dog at 15 cm. Homologous parts labelled as in figures 1 and 3. A light line has been traced through the record. The marks below the base line indicate the time in σ. X = 82 mm., 5000ω, body temperature. Natural size.

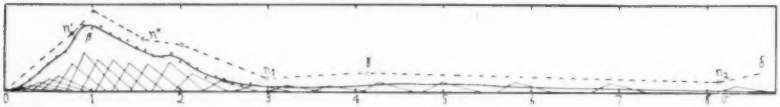

Fig. 5. Reconstruction (solid line curve) of the action potential in the saphenous nerve at 15 cm. The points joined by the dotted lines represent accurately in time, but arbitrarily in amplitude, the corresponding points in figure 4, the start of figure 4 coinciding with the start of the reconstruction. Designations same as in figures 1 and 2.

Here again, the beginning of the last wave of the recorded action potential coincides with the beginning of the axon action potential in the 3.0 to 3.5μ group of fibers. Whether the notches, n_1 and n_2, that appear in the first large wave are of any significance it is impossible to say. However this may be, we shall for the present regard the first large wave in the saphenous action potential as a unit.

If the muscle nerve and saphenous nerve fiber-size counts were combined (see fig. 2) the result would be a diagram, consisting of a continuum of fibers presenting three definite crests, essentially the same in appearance as the diagram obtained through the analysis of the fibers of a mixed nerve (compare, for example, with figure 6 of Gasser and Erlanger (1926)); and addition of the reconstructed muscle and saphenous action potentials

to each other ($M + S$, fig. 3) produces a three-waved action potential almost exactly the same in appearance and in time relations as the action potential obtained from the tibial nerve of the dog (pictured as fig. 14, B' in a previous publication (1924)).[2] It may be concluded, therefore, that the pile of fibers that produces the first wave in the saphenous action potential forms in mixed nerves, such as the tibial and sciatic, the second wave. If, in any given nerve, the designation of the waves is to be based upon homologies, rather than upon the order of their conduction rates, which had been the method heretofore used, it would seem, therefore, to follow that the tibial, the sciatic and presumably the femoral action potentials are composed of *alpha, beta, gamma* and *delta* waves, the saphenous of *beta, gamma* and *delta*.

Here may be presented such evidence as we have indicating that in mixed warm-blooded nerve the grouping of fibers that leads to the production of waves in the action potential has a functional significance. Previous work (1926) has shown that the *alpha* wave in the sciatic action potential in reality is double, being constituted of two parts, one contributed by the fibers from the motor root, the other by the first of the three or four waves developing in the fibers from the sensory root; these two waves, though, travel at almost exactly the same rates and consequently remain in phase as they move away from the site of stimulation. Present observations, in showing that the α wave of a mixed nerve passes into muscle branches but not into skin branches, indicate that the sensory component of the α elevation is produced by fibers mediating muscle sense. This conclusion bears out Sherrington's finding (1894) that the large sensory fibers of a mixed nerve run to voluntary muscles.

Muscle is endowed not only with muscle sense, but with other senses as well; and Sherrington (1894) has shown that the small fibers of muscle nerves come largely from the posterior roots. We have no means of knowing what may be the range in size of the muscle sense fibers or what the range of the motor fibers. A study of the anterior roots of the bullfrog (see fig. 2, 1927) makes it clear that the motor fibers range down from 20, certainly to 12μ, and probably to 10 or even 8μ. Obviously the motor fibers, and this probably is true also in the case of the muscle sense fibers, extend well beyond the cumulus that they produce in the fiber-size pattern. Furthermore, it is not impossible that the several senses are mediated by fibers whose diameters in each case range between certain, perhaps rather wide, limits, but nevertheless are massed so as to produce, together with the motor fibers, the characteristic fiber-size patterns. On the basis of

[2] The conduction rate in the tibial was slower (87 m.p.s.) than in the present femoral preparation (92 m.p.s.), but the distance of conduction was greater (8.2 cm. as compared with 7.0 cm.), so that the two records are comparable practically without change.

this hypothesis it will be convenient to designate the several senses and the corresponding fibers noncommittally as *alpha*, *beta*, *gamma* and *delta*. It is worth noting in this connection that in the fiber-size pattern of the muscle nerve there are only very slight indications of accumulations of fibers in positions corresponding with the two crests exhibited by the saphenous pattern (see fig. 2). Possibly this may be taken as evidence that though muscle, like skin, is supplied with the "*beta*" and "*gamma*" senses these are relatively feebly developed there. Furthermore, muscle probably is supplied with the sense that is mediated by the unmyelinated fibers (the "*delta*" sense), though concerning this we have no direct evidence.

This study of mammalian nerve fails to give any clue to the functions of the *beta*, *gamma* and *delta* sense fibers. It is rather suggestive, however, in view of the general tendency to recognize three groups of skin senses, namely, pressure, temperature (warmth and cold) and pain, that analysis of a skin nerve on the basis of the sizes of its constitutent fibers, discloses three aggregations of fibers. There is considerable evidence, which is reviewed by Ranson (1921), indicating that the unmyelinated fibers in the lateral division of the posterior root mediate, among other things, pain. Presumably, therefore, the *delta* sensory fibers are concerned with pain. On this basis the *beta* and *gamma* fibers would remain for the mediation of the touch and temperature senses. We wish to emphasize, however, that this assignment of functions to the four sensory fiber groups apparently contained in mixed nerve is to be regarded merely as tentative. At this stage of the investigation we can affirm only that the *alpha* wave of the action potential of mixed nerve is produced by the larger fibers, and that these are mainly motor and muscle sense in function.

The inferences derived from the foregoing comparison of the histology of the branches of the femoral nerve of the dog with their action potentials are supported and broadened by similar studies on skin nerves of the bullfrog. The nerves crossing the dorsal lymph sac, along with blood vessels and connective tissue, though only 2 to 3 cm. in length, have been found to yield small but perfectly definite action potentials rather constant in configuration. Typically, the records (fig. 6) show two waves, of which the second is higher than the first. The rate of propagation of the wave front ranges in the different preparations between 26 and 35 m.p.s.

The histological analysis of one of these skin nerves is shown in figure 7. These nerves are exceedingly fine and it was, therefore, convenient to enlarge the section sufficiently (the photograph measured was a 3000 fold enlargement) to bring out fairly clearly the very smallest fibers. One could see in the enlargement even the unmyelinated fibers, outlined by their thin lipoid membranes, though it was not possible to measure them accurately. However, they were counted, and have been so placed, as

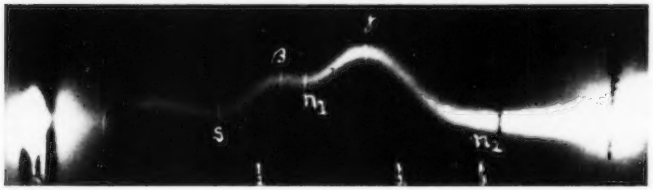

Fig. 6. Record from a dorsal skin nerve of the bullfrog at 19 mm. Deflection prior to *S* is the escape. Room temperature. X = 86 mm., 2000$^{\sigma}$. Natural size.

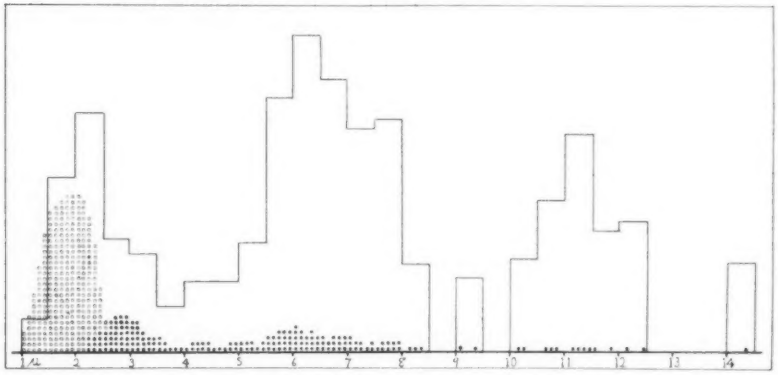

Fig. 7. Fiber-size pattern of all of the fibers in a dorsal skin nerve of the bullfrog. Dots represent measured diameters, circles those estimated; most of the latter represent unmyelinated fibers, and are somewhat uncertain as to number and arrangement.

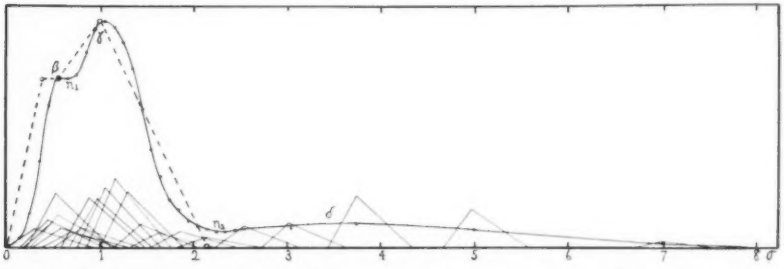

Fig. 8. Reconstruction (solid line curve) of the action potential in a dorsal skin nerve of the bullfrog at 19 mm. The circles joined by the dotted lines represent accurately in time the corresponding points in figure 6.

circles in the diagram, as to make a uniform pile with its limits set as accurately as possible in their proper locations. This part of the diagram, therefore, must be regarded merely as a rough approximation.

Figure 8 is the action potential reconstructed on the basis of this analysis, taking 19 mm. as the distance of conduction (that is, the distance of conduction obtaining in the case of the action potential shown as figure 7) and 42 m.p.s. as the conduction rate in a 20μ fiber (that is, the usual size of the largest fiber of, and rate of conduction in, the sciatic nerve of the bullfrog). On the basis of the relation of fiber size to conduction rate, the action potential in the largest fiber of the skin nerve under these conditions should travel at the rate of 29.3 m.p.s., which, as has been seen, is about the usual conduction rate in these skin nerves. It is a bit faster than the rate at which the β wave moves in the sciatic of the bullfrog.

The measured times from the start of the action potential to the significant points on the record have been placed as circles in figure 8, each at the height of its corresponding point in the reconstruction, and these points have been joined by dotted lines. The very close resemblance of the reconstruction to the record thus is made obvious. As in the case of the femoral nerve, the recorded features here also are just a bit too early. The reconstruction would lead one to expect in the recorded action potential a third wave reaching its maximum about 3.6σ after the start. The record gives little, if any, evidence of such a wave; but if there were one, and if it had the same relative height that it has in the reconstruction, namely, about $_1{}^1{}_2$ that of the main wave, it scarcely would be discernible in the record.

In the light of the results obtained through the comparison of the action potentials in the muscle and saphenous branches of the femoral nerve, and in view of the relative rates of conduction in the dorsal skin and sciatic nerves of the frog it seems justifiable to conclude that the first wave in the skin nerve of the frog is the homologue of the second or *beta* wave in the frog's sciatic nerve, and that the skin nerve, like the saphenous nerve, contains the *beta*, *gamma* and *delta* groups of fibers, but not the *alpha* group.

The greater prominence of the second wave in the action potential of the skin nerve of the frog than in that of the skin nerve of the dog is, of course, the expression of the relatively greater number of fibers in the second pile in the former nerve; but this fact possibly indicates that the "*gamma*" sense is better developed relatively in the frog. Since the skin of the frog is bare, while that of the dog is hair-covered, and since a bare skin probably would be supplied with a greater number of temperature receptors than a covered skin, and since, furthermore, one of the several types of tactile organs is found in association with hair follicles, it seems justifiable to venture the guess that the "*beta*" sense is touch, the "*gamma*" sense,

temperature. In any event it would prove interesting if, through the histology of nerves, one could obtain some conception of the sense experiences of animals.

SUMMARY

Comparison of the fiber-size patterns with the action potentials in muscular and cutaneous branches of the dog's femoral nerve and in skin nerves of the bullfrog confirms previous observations proving that the conduction rate in the fibers of a nerve is approximately directly proportional to the diameters of its fibers, and that the grouping of the fibers of a nerve according to their sizes determines the configuration of its action potential.

Corresponding with the four waves in the action potential of a mixed nerve there are in the fiber-size patterns of such a nerve four aggregations in the continuous range of fibers, of which three are myelinated. Of the branches of the femoral nerve, those supplying voluntary muscle exhibit one very definite pile of fibers, which form the sole visible wave (α) in their action potentials, and three indefinite accumulations (if unmyelinated fibers be included); the branch supplying the skin (the saphenous nerve) exhibits three cumuli and the corresponding waves, namely, *beta*, *gamma* and *delta*, in its action potential. The *alpha* fibers, in the sense in which they are defined herein, convey motor and muscle sense impulses. The *delta* sensory (probably unmyelinated) fibers presumably mediate pain. On this basis the *beta* and *gamma* fibers would be left for the mediation of touch and temperature impulses.

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DELAY OF BLOOD IN PASSING THROUGH THE LUNGS AS AN OBSTACLE TO THE DETERMINATION OF THE CO_2 TENSION OF THE MIXED VENOUS BLOOD

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Various procedures have been developed for the purpose of getting a sample of lung air that has come into equilibrium with the mixed venous blood, which enters the lungs from the right heart.

The importance of getting the CO_2 (or O_2) tension of the mixed venous blood, relates itself to the fact that it is possible, by the principle of Fick, to calculate the cardiac output, if we know the CO_2 output and the difference between the CO_2 tensions of the arterial and venous blood. (For literature see Henderson (1) and Wiggers (2).) The CO_2 tension of the arterial blood may be considered the same as that of the alveolar air, and if we know the tension of the mixed venous blood, we can calculate the CO_2 that a liter of blood will bring to the lungs, and hence the cardiac output either from the CO_2 dissociation curve of normal blood or better perhaps, from experiments upon the blood of the subject himself. It is relatively easy to get the CO_2 output and it is possible (3) to prove that the alveolar and arterial CO_2 tensions are similar. However, that any of the rebreathing procedures in vogue give us a CO_2 tension similar to that of the mixed venous blood, is an assumption that is not backed up by any direct experimental evidence and, as will be seen in the light of evidence adduced herewith, is a rather dubious assumption.

Christiansen, Douglas and Haldane (4) record the interesting finding that when the breath is held for varying lengths of time, there is a continuous increase in the CO_2 tension of the alveolar air. The experiments were carried out with the idea that the lung air might quickly reach equilibrium with venous blood, which would show itself as a plateau in the ascent of the CO_2 tension.

The absence of any sign of such a plateau could be explained on the assumption either (*1*) that during the time the breath was held, the average tension of the blood in the lungs increased slowly, or (*2*) that the tension of the lung blood changed at once from arterial to venous, but the lung air,

due to slow diffusion failed to register this tension until after the lung blood had been contaminated by recirculated blood.

The latter of these hypotheses seems to be the basis for the various rebreathing procedures which have developed since this observation was made. It is to be looked at askance since CO_2 diffuses through the animal membrane twenty or thirty times as rapidly (5) as does oxygen, and there is no very good reason for expecting the CO_2 tension of the lung air to be markedly different from that of the lung blood, as long as there is fair oxygenation of the arterial blood. Evidence which confirms this attitude is adduced by Meakins and Davies (6) who have shown that then the breath is held for forty seconds, there is a much greater disturbance in the relation between arterial and alveolar oxygen than is the case for CO_2.

It is implied in the paper of Christiansen, Douglas and Haldane that they regarded the continuous rise in the alveolar CO_2 tension after breath holding, to be due to failure of the lung air to equilibrate with the lung blood before re-circulation. The result of this attitude was that these workers elaborated a method for using the lungs as an aerotonometer and by "straddling" the supposed venous tension, could approach it from above or from below, and be quite sure of the CO_2 tension of the blood in the lungs. Their experiments gave results that were no doubt of the right order, for the CO_2 tension of the blood in the lungs, at the end of the experimental time, but since there was no attempt to show that the tension remained stationary, the experiments cannot be taken to prove that the tension of the blood in the lungs equalled that of the mixed venous blood.

Henderson and Prince (7) simplified the procedure of estimating the CO_2 tension of the lung blood, by rebreathing for ten seconds, some expired air. After waiting until the circulation and respiration had reached normal, they repeated the procedure until the CO_2 tension of the repeatedly rebreathed air had reached a constant value. When these investigators varied the procedure by commencing a similar experiment with a nine per cent mixture of CO_2, and came down to the same value they had arrived at from below, they thought that they had proof that the CO_2 tension of the rebreathed air was approximately that of the venous blood.

This argument is rather dangerous because it fails to take into account the fact that at each rebreathing, the lung-bag mixture is diluted by 1000 to 1500 cc. of residual air which has the alveolar rather than the venous tension. This dilution alone would produce a lowering of the CO_2 tension in the lung-bag mixture, making the second experiment a mere repetition of the first, for in each case, after the tension has reached a constant value, the residual air dilutes the lung-bag mixture at the beginning of the rebreathing, and the final value is approached from below and not from above, even though a few minutes before, the CO_2 tension had been 9 or 10 per cent of an atmosphere in one bag and 3 per cent of an atmosphere

in the other. Later work has modified the procedures of Christiansen, Douglas and Haldane and those of Henderson and Prince, but has not changed them in principle.

We can no longer ignore the hypothesis that when the CO_2 tension of the inspired air is increased, there is a gradual rise in the average tension of the lung blood. Drinker (8) has brought together evidence that the passive regulation of the vascular bed in the lungs, is such "that when the blood flow through the lungs is rapid, all available vascular routes are conducting blood and are somewhat distended. When the flow falls and the pressure playing upon different capillary paths is low, then only the easiest routes transmit the effective blood current, and blood moves sluggishly through the more difficult." These latter conditions exist probably during rest, since the vascular bed of the lungs is capable of carrying a fivefold increase in flow during exercise. Drinker also adduces unpublished evidence indicating "a tailing out of columns of injected solutions as they pass through the lungs in man." We are reporting elsewhere experiments which confirm this.

If we look at the pulmonary circulation in this way, we are led to the following hypothesis:

When one starts to rebreathe "virtual venous air" the blood in the lungs is not wholly venous, but more or less arterial in some parts and venous in others, and the tension of the lung air is an expression of the average of these various tensions. This average constantly changes as the arterial blood enters, and is further changed as re-circulated blood begins to come back to the lungs. If this attitude is correct, we are not justified in determining the venous CO_2 (or O_2) tension by any of the usual procedures which use the lungs as an aerotonometer.

To test this hypothesis, we endeavored to find out whether there was a low CO_2 tension in the lung air when rebreathing had been carried out for a short time, to correspond with the assumption that early in the experiment, there is a great deal of arterialized blood in the lungs. We tried also to find out whether this CO_2 tension gradually increased as the rebreathing time was lengthened to correspond with the assumption that this arterialized blood was gradually replaced by venous blood entering the lungs from the right heart.

1. *Relation between CO_2 in rebreathed air and time of rebreathing.* The apparatus consisted of the "tensimeter" described by Henderson and Haggard (9) modified in that the bag containing the mixture of CO_2 and O_2 was on the end of a thickwalled, soft rubber tube, similar to that used for the Haldane-Priestley alveolar samples. This permitted getting samples of air which had certainly come from the depths of the lung. Samples were taken in oiled 20 cc. Luer syringes, connected by short, soft rubber tubes to needles which were thrust through the tube. The sam-

pler tubes were clamped with hemostats and the samplers were found very convenient in that they seldom leaked, and were easily tested for leaks, that the dead space was small and easily washed out, that it was extremely simple to deliver the sample to the Haldane-Henderson analyzer, and that we got satisfactory duplicates. The rubber tube from which the samples were taken was of such consistency that the needle holes closed against many times the pressure which occurred during an experiment.

The procedure consisted in filling the bag with 2.5 l. of a 6 per cent mixture of CO_2 in oxygen. After resting for ten minutes to half an hour with the mouth piece in place, the subject rebreathed this gas mixture twice in six seconds. After three minutes, he rebreathed it again in a similar fashion. This was repeated seven times. Samples were taken after the fourth, fifth, sixth and seventh rebreathings. Occasional oxygen analyses

TABLE 1

Relation of the difference between the CO_2 tension of alveolar and rebreathed air

REBREATHING TIME (SECONDS)	DIFFERENCE IN MILLIMETERS CO_2 BETWEEN ALVEOLAR AND REBREATHED AIR	DIFFERENCE IN PER CENT OF THE 15 SECONDS VALUE CO_2 TENSION BETWEEN ALVEOLAR AND REBREATHED AIR
CO_2 not added between rebreathings		
6	6.6 ± 0.13	74 ± 1.5
9	7.3 ± 0.12	82 ± 1.4
12	7.8 ± 0.09	87 ± 0.8
15	8.9 ± 0.13	100 ± 1.5
12–15 cc. CO_2 added between rebreathings		
8	7.9 ± 0.11	79 ± 0.9
16	8.9 ± 0.13	100 ± 1.5
24	10.4 ± 0.13	117 ± 1.5

were made of the seventh sample to assure ourselves that there was enough oxygen in the lungs to saturate the hemoglobin. Occasional Haldane-Priestley samples of the alveolar air were taken.

Using fresh bagfuls of the same mixture, the rebreathing was repeated on the same subject at the same sitting, using exactly the same procedure except that he breathed three times in nine seconds. This series was followed by three others in which the rebreathing was four times in twelve seconds, five times in fifteen seconds, and a repetition of the first series, two times in six seconds. This whole procedure was repeated with sequence variations three or more times on each of the three collaborators, and was controlled by increasing the rebreathing time without increasing the rebreathings to eliminate the effect of re-exposure of dead space air to the alveolar epithelium.

The results of these experiments are shown in figure 1 and in table 1, and were calculated as follows: The difference between the tension of the average alveolar air for each experimental series and all of the fifteen second re-breathed air samples were tabulated and the mean with its probable error calculated. The same was done with all of the twelve, nine and six second values.

The figures indicate 1, since the differences are several times their probable error, they are "almost certainly significant;" 2, that the re-

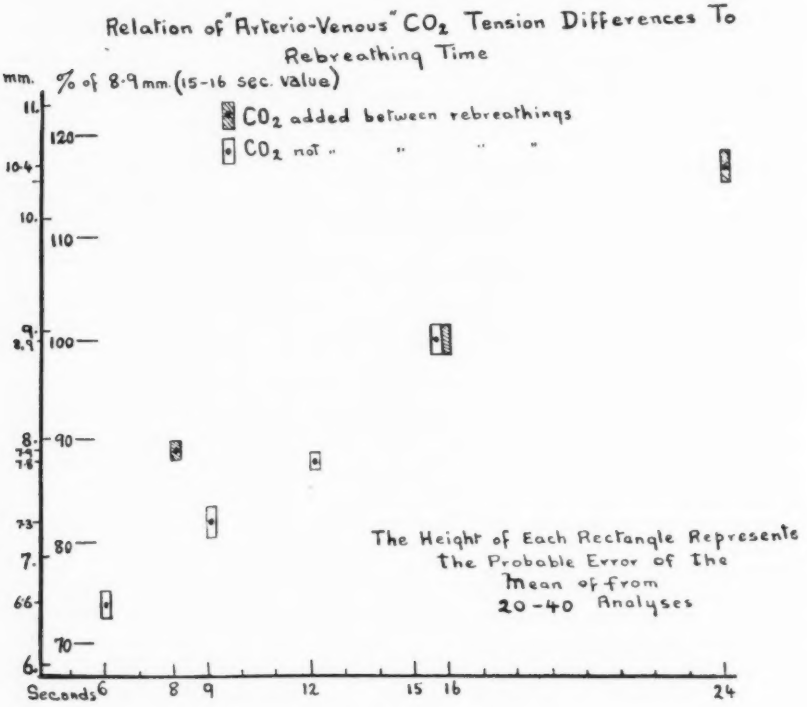

Fig. 1

breathed air and hence probably the lung-blood increases its CO_2 tension steadily as the rebreathing time increases; 3, since the cardiac output is calculated by multiplying the CO_2 difference between the alveolar and rebreathed air by a figure which represents the CO_2 dissociation of normal oxygenated blood, and dividing the answer into the CO_2 eliminated, hence changes in the rebreathing time affect the cardiac output figure to the

same proportionate extent as they do the figure representing the difference between the alveolar and rebreathed air, i.e., increasing the rebreathing time from six to fifteen seconds, decreases the "cardiac output" figure by 25 per cent.

2. *Control experiments ruling out slow CO_2 diffusion.* The gradual increase in the tension of the rebreathed air might be due to slow diffusion of CO_2 from wholly venous blood in the lungs. Reasons for doubting this have already been mentioned, and in addition, the following experiments show conclusively that the changing tension of the lung blood is responsible for the changes in the rebreathed air. The experiments were carried out very much as before except that 12 to 15 cc. of CO_2 were added before each rebreathing. The rebreathings were two in eight seconds, four in sixteen seconds, and six in twenty-four seconds. The series were as large as in the previous experiments, and carried out in similar sequence. The addition of CO_2 serves to compensate for the lowering of the CO_2 tension by dilution with residual air, and insures that diffusion shall, at all times, be inward from lung air to lung blood.

The eight second is well below the sixteen second value, but somewhat above the nine second value of the previous experiment. This is to be expected since diffusion is at this time inward into the lung blood.

The sixteen second is about the same as the fifteen second value when no CO_2 is added. This corroborates a statement found in the paper of Field, Bock, Gildea and Lathrop (10) to the effect that the same equilibrium is reached in experiments lasting fifteen seconds, whether CO_2 is added or not.

The same is, of course, true for the twenty-four second value which is definitely higher than the sixteen second value.

In discussing a report of these findings at Rochester, N. Y., Professor Y. Henderson suggested that a level representing the venous tension might be arrived at by rebreathing for a longer time. To test this hypothesis the results of rebreathing for twenty-four and thirty-two seconds were compared. The difference between the alveolar and rebreathed air was 1.55 millimeters (15.0 per cent) higher at the end of thirty-two seconds re-breathing than at the end of twenty-four seconds. This rise in CO_2 tension can only be attributed to re-circulated blood.

3. *Quantitative proof that CO_2 diffuses into the arterialized blood in the lungs.* To arrive at a more quantitative proof that arterialized blood in the lungs is responsible for the low CO_2 tension in the shorter experiments, we calculated the quantity of CO_2 in the lung-bag system at various stages of a typical rebreathing procedure, to find out whether CO_2 actually leaves the lung air for the lung blood.

First the residual air was estimated in the following manner: Oxygen was breathed until the atmospheric nitrogen was ventilated out of the

alveoli of the lungs. Four liters of N_2 were then rebreathed until mixing was complete (11), and the residual air was calculated from the proportion of nitrogen in a sample taken from the bag. Checks were satisfactory and the analyses could be carried out without modifying the ordinary analytical procedures.

Two and five-tenths liters of "virtual venous" air (16 sec. value) were placed in a bag, connected by a long rubber Y tube to the inspiratory and expiratory sides of a valve with very small dead space. During inspiration

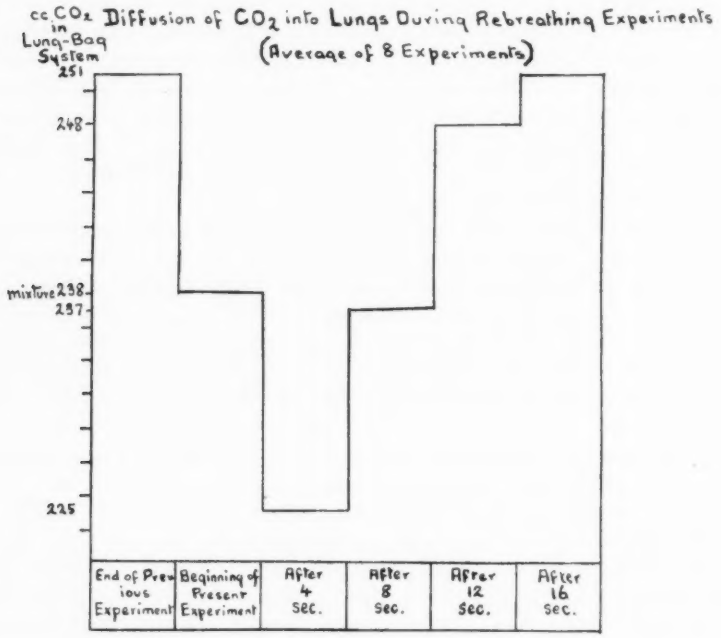

Fig. 2

from the bag it was possible to collect, from the expiratory tube near the valve, a sample of the last expired air from the depths of the lungs, and from the inspiratory tube a mixed sample of the air that was contained in the bag. Four samplers were connected to the inspiratory and four to the expiratory tubes of the valve, which was in turn connected to the "venous" opening of the "tensimeter." A sampler was connected to the Haldane-Priestley tube of the "tensimeter."

While the subject was breathing normally, the "tensimeter" was turned

to the H-P tube, and he was instructed to expire forcibly. At the end of this expiration the "tensimeter" was turned to the "venous" opening, connected as described with the small respiratory valve. He then inhaled from the bag for two seconds and exhaled again into the bag for two seconds. The subject had a stop-watch and repeated these carefully timed respiratory movements until he had executed four inspirations and four expirations in sixteen seconds. During each inspiration after the first a sample of the mixed bag air was taken from the inspiratory tube and a sample of the deep lung air from the expiratory tube.

From the amount of air in the bag and in the lungs (residual air) and their respective CO_2 percentages (alveolar analysis and virtual venous analysis) was calculated the total amount of CO_2 in the lung-bag system before the first breath. The average value for eight experiments was 238 cc. (fig. 2).

Using the first mixed-bag sample, the CO_2 content of the lung-bag system was again calculated. It was found to average 225 cc., i.e., 13 cc. less than there was at the beginning. Thus all of the CO_2 which enters the lung with the venous blood, as well as 13 cc. of the inspired CO_2, have disappeared altogether.

Analysis of the second sample showed that all of the CO_2 brought in by the venous blood during the first eight seconds of the experiment as well as 1 cc. of the inspired CO_2 had disappeared.

It is difficult to think that this CO_2 can go anywhere but into the arterialized blood which remains in the lungs.

The next samples at twelve and sixteen seconds show that the CO_2 content of the system is gradually coming back to what it was at the end of the experiment in which the "virtual venous air" was prepared,—a a duplicate of the one we have just analyzed in detail.

The samples taken from the expiratory tube of the deep lung air served as a check as to mixture and direction of diffusion.

Discussion. The fact that the "virtual venous" CO_2 tension gradually increases as the rebreathing time is lengthened, introduces a systematic error that may amount to forty per cent in the cardiac output figure. That such a source of error should have remained unnoticed is surprising until one considers the variability of the results even when the experiments are carried out most carefully and the procedures are exact repetitions of each other.

It is quite common in our experience and in the literature to find a single series of rebreathed air analyses that shows random fluctuations of a millimeter or more in CO_2 tension. A fluctuation in the venous tension, say from 49.0 to 50.0 mm., is unavoidable. Since it is unavoidable one finds oneself regarding it as a mere 2 per cent variation. In reality it changes the difference in tension between the alveolar and rebreathed air,

and hence the cardiac output figure by 10 to 15 per cent. These 10 to 15 per cent variations would obscure any but an exhaustive attempt to check up on the relation between rebreathing time and "virtual venous CO_2 tension."

SUMMARY AND CONCLUSIONS

The difference between CO_2 tension of alveolar and of rebreathed air increases significantly as the rebreathing time increases.

This seems to result from the fact that at the beginning of a rebreathing experiment, the lungs are full of arterialized blood. As this blood leaves the lungs and venous blood enters, the tension of the rebreathed air gradually rises as the mean tension of the blood in the lungs rises. This rise continues as re-circulated blood enters the lungs.

These facts cast doubt upon all methods of calculating the circulation based upon the use of the lungs as an aerotonometer for CO_2. The same difficulties would apply to oxygen but might not apply to nitrogen or the "foreign" gases.

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CENTRAL FACTORS IN HUNGER

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According to the observations of Cannon and Washburn (1912) and of Carlson (1913, 1916), hunger is mainly of gastric origin. L. R. Müller, however, maintains that hunger is of central origin (1915, 1924, 1926a, 1926b). He believes that a depletion of readily available food reserves in the blood affects the hunger center and that this gives rise to gastric contractions which, in turn, give rise to sensations that are recognized as hunger. This explanation fails to account for the periodicity of the gastric hunger contractions. Moreover, Carlson produced gastric contractions by local stimulation in a subject with a gastric fistula. These contractions were experienced as hunger although they were not of central origin.

The present claim that central rather than gastric factors determine hunger rests primarily upon evidence that the periodic gastric contractions may occur without giving rise to hunger sensation and hunger may be experienced independent of these contractions. Rupp, for instance, found that mild fever (100° to 102°F.) did not appear to alter the gastric hunger contractions, but instead of giving rise to hunger sensations, the contractions then produced headache, nausea and an epigastric distress (Carlson, 1916). Similarly, Meyer (1918), observing tuberculous subjects, found that most of them had essentially normal periods of gastric contractions, but instead of being recognized as hunger sensations, they were described as restlessness, headache and other symptoms.

Under more normal conditions, a lack of correlation between gastric contractions and hunger has also been observed. Boldyreff (1916) claimed that the periodic gastric contractions gave rise to sensations but not to hunger and Anitschkow (1924) seems to hold a similar view. Iwanow (1927), using 24 subjects, reported that in most cases the periodic gastric contractions were not associated with hunger. Carlson (1914, 1916, 1918) found that the hunger sensations may decrease during starvation even though the periodic contractions increase. Carlson (1913, 1916) also noted that the first period of hunger contractions after a meal was usually felt less keenly than subsequent periods. Was not this due to the

effect of the recent meal on hunger? That this is the explanation is made practically certain by experiences of my own like the following:

February 28, 1924. Having previously been inclined to eat most of my food in the evening, I began a trial of eating the bulk of my food early in the day. Had most food before noon. Retired early to avoid an expected temptation to eat again later in the evening.

February 29. Rather hungry early. Had most food, including 12 eggs, before 11:00 a.m. Felt full to the point of considerable discomfort but not satisfied. During the afternoon, confined myself mainly to the use of sweetened drinks. After 7:00 p.m., with a bar of almond milk chocolate within arm's reach, felt no desire whatever to eat. Distention in the gastric region entirely gone. About 10:00 p.m., thought that diarrhea was developing when cramp-like epigastric pains came on. Realized that I had experienced a period of gastric contractions when it ended with gastric tetany. Had no desire to eat. No desire to defecate after the period ended. This proved that the pains were not sensations commonly associated with diarrhea and previous experience with the balloon method also made it possible to recognize the periods of gastric contractions subjectively. Went to bed when another gastric period without hunger developed about an hour later.

March 1. Woke at 5:30 a.m. in the midst of a gastric period *which involved less local (epigastric) pain than the periods of the preceding evening but which was accompanied by a rather vague desire to eat.* Did not care for much food—evidence of having eaten more than enough for one day the day before.

In this and in similar subsequent experiences the desire to eat was evidently suppressed as a consequence of the absorption of food and hunger sensations therefore did not develop with the gastric contractions until some degree of a need of food was again occasioned. Sometimes, however, a dietetic excess was found to delay assimilation by retarding gastric evacuation. Or, the periodic gastric contractions would be delayed in onset. Or, again, the contractions would give rise to no sensations and could then be observed only by the balloon method.

These findings have helped to explain some previous experiences. For instance, when I ate most of my food in the evening, a complete but otherwise normal indifference to food was sometimes observed on the following morning. It was then assumed that the intervening sleep somehow accounted for this but it is now clear that a central repletion resulting from the ingestion of a large quantity of food was the main factor. However, when records of hunger contractions were taken under conditions that were supposed to be similar (Carlson, 1918), a complete lack of a desire to eat was never noted. This was evidently due to curtailing my food intake because of being under observation. Nevertheless, the eating of all of my food in the evening left me with so little desire to eat when some of the gastric contractions were registered that I could not regard them as a true index of hunger (Carlson, 1918—appendix). The "bulimia" referred to in that study was undoubtedly due to a keen hunger incident to central depletion which was occasioned by the deliberate abstinence

from food earlier in the day. This hunger naturally persisted even after the stomach was more than commonly filled and decreased only after some of the food was apparently digested and assimilated. This was not merely a persistent appetite (regarded as a call for food which is particularly relished) for the tendency at such times was to eat even unpalatable food when nothing more suitable to the taste happened to be immediately available. Moreover, the "bulimia" disappeared whenever food was taken more frequently. In fact, I found since then that by using food which is easily digested and using most of it early in the day, I could satisfy hunger daily for several months in succession without eating enough to maintain weight or energy.

The manifestation of hunger independent of epigastric sensations and failure to recognize this also seems to explain the otherwise paradoxical claims that hunger disappears with prolonged fasting. In the first place, such claims mean only that those sensations which the subjects, *under ordinary circumstances*, learned to regard as the indication of hunger disappeared. Carlson observed that the periodic gastric contractions tend to increase with starvation and graphic records of them have been obtained on man as late as the 40th day of fasting (Hoelzel and Kleitman, 1927). But the sensations due to these contractions were found to change, decrease or even disappear. At the same time, the desire to resume eating more or less progressively increased. At least this has been a constant finding in my experience of fasting from 1 to 41 days (now totaling over 500 days) and a consideration of the behavior of others who have fasted indicates that they were similarly affected. The increasing tendency to resume eating, rather than the epigastric sensations which are ordinarily regarded as hunger, therefore seems to point to the true nature of hunger.

Considered in this light, hunger, in one sense, is not a sensation at all but rather a condition involving a more or less compelling tendency or *urge to do something*—to eat. It is more nearly a motor than a sensory phenomenon and is something like, but naturally less imperative than, the impulse to breathe (Müller). Like other drives, it yields pleasure in appropriate action, while restlessness and dissatisfaction are occasioned when it is thwarted. In so far as hunger is a sensation, it seems to be a general sensation (not localizable). In conformity with this analysis, appetite appears to be hunger or mild hunger plus taste-memory processes.

Satiety must evidently be viewed in two ways; first, as a reflex inhibition of hunger from the alimentary canal and, second, as a true satisfaction of hunger in consequence of the absorption of nutriment. Hunger, when not extreme, is reflexly inhibited as a result of the filling of the digestive tract and apparently also because of changes in the gastro-intestinal chemistry. Ordinarily, hunger is reflexly only diminished and a mild hunger, commonly regarded as appetite, persists. However, when hunger

is completely satisfied as a result of the absorption of food or when it is suppressed by strong reflex inhibition or by humoral (toxic—?) conditions, this "appetite" also disappears.

Kestner (1919) contends that the amount of gastric juice secreted and the length of its stay in the digestive tract (delay in reabsorption of the gastric juice) determine the satisfying value of various foods. He particularly extols the satisfying effect of meat. However, my own observations lead me to attribute the soothing effect of meat (or other protein) mainly to its power to reduce the gastro-duodenal acidity. First it serves to bind the acid and next it tends to keep the acidity within moderate bounds by "fatiguing" the gastric secretory mechanism (Hoelzel, 1926). Finally, the digestion products of protein also seem to have a specific central satisfying effect.

The gastro-duodenal acidity appears to play a considerable rôle in determining the common reference of hunger to the periodic gastric contractions. Over 3000 aspirations of the fasting stomach made upon myself indicate that the acidity of the gastric secretion is highest during the periods of contractions and lowest during motor quiescence. This variation alone however means little as the acid seems to give rise to sensation in the duodenum rather than in the stomach (Hoelzel and Kleitman, 1927). But the acid is passed into the duodenum at its highest concentration with the periodic gastric contractions, to which the sensations due to the acid are therefore likely to be attributed. The hunger-nausea of some individuals may be a consequence of the acid irritating a very sensitive duodenum. In my own case, the sensation ascribed to the acid closely resembled hunger of central origin in its diffuse nature and also in being more uniformly accompanied by a keen desire to eat than the sensations which were more directly attributed to the gastric contractions. A specific sensibility of the digestive tract nevertheless was thought to be a greater factor than the gastric acidity in giving rise to distinct sensations (Hoelzel and Kleitman). An increased permeability of the gastro-intestinal mucosa was believed to account for this peculiar sensibility but part of it, and particularly the early phases, must evidently be attributed to changes in central conditions. Carlson (1916) already suggested such changes as explaining some of the variations in sensation observed with the gastric contractions. In the absence of the specific gastro-intestinal hypersensibility or the central hunger conditions, acid normally seems to give rise to no sensation whatever or only to burning sensations.

A further cause of the reference of hunger to the gastric region is seen in the relation of the periodic gastric contractions to gastric emptiness. Obviously, hunger is more likely to be manifested with an empty stomach than with a full one but a periodic filling and emptying of the stomach also takes place in conjunction with its alternate motor quiescence and

activity during fasting. That is, the continuous or fasting gastric secretion, together with swallowed saliva and air, accumulates in the stomach during motor quiescence and passes out with the periodic motility (Hoelzel, 1925). (The air or gas is forced out last and gives rise to the hunger-borborygmi.) Consequently, one feels particularly empty whenever the periodic contractions occur. On the other hand, I found that the filling of the stomach between these intervals sometimes reaches a point where a distinct sense of agreeable fulness is felt. The periodic gastric contractions are therefore aptly referred to in the German literature as empty-contractions (*Leercontractionen*) and the resulting sensations, independent of hunger, might be well described by a similar term.

However, although the feeling of emptiness helps to refer hunger to the stomach, a more fundamental reason for this association evidently is the fact that hunger due to central conditions is ordinarily present when the periodic gastric sensations are manifested. But the epigastric sensations are often disagreeable and usually develop suddenly while hunger due to central factors is not inherently disagreeable and the exact time of its onset defies introspective analysis. Consequently the more outstanding sensations in the gastric region are likely to be accepted as the index of hunger. Likewise, headache, weakness, mild nausea or other symptoms may come to be regarded as signs of hunger when they are regularly associated with it.

Nevertheless, by separating the incidental from the indispensable phenomenon, the essential element in hunger is seen to be the central condition. Such an explanation of hunger can be applied to animals in general from the amoeba to man. With this, the inhibition of hunger in the bull seal and salmon during the seasons of sexual excitement becomes less of a mystery. We also see why animals deprived of their stomachs continue to eat. Moreover, the effect of fever, emotions or intellectual interests in suppressing hunger seems more understandable when central rather than gastric determinants of hunger are granted.

If we next consider whether hunger is more directly dependent upon the nutritive condition of the tissues or cells in general (Turro') or of the major food reserve depots such as the liver and muscles (Shur, Brugsch) or of the blood (Dresel and Rothmann, Müller), the latter seems to be indicated. That the state of nutrition of the cells in general does not directly determine hunger was apparently proven in my experience when a few meals after fasts of from 15 to 41 days brought about a state of complete satiety. To be sure, the satiety was then of relatively short duration but this could be accounted for by the avidity of the starved cells in robbing the blood of any surplus nutriment. On the other hand, obese individuals may be hungrier than undernourished subjects and this again shows that the general state of nutrition does not directly affect hunger in the complex

organisms. It is likewise doubtful whether the depleted state of the liver and muscles directly gives rise to hunger. Biedl, Brugsch, Durig, Mark and Wagner and Shur apparently could not accept the view that the blood state determines hunger in man because a diabetic with a high blood sugar may be very hungry. But since a high blood sugar is of no nutritive value to a diabetic with pancreatic (insulin) deficiency, it also may not affect the hunger center in a normal way. Normally, the blood sugar level evidently affects hunger (Mark and Wagner, 1925) and incidentally it influences the periodic gastric contractions (Bulatao and Carlson, 1924). Thus it serves as a link between central conditions and gastric sensations. Nevertheless, the blood alone is not a sufficiently great nutrient reserve depot to account for all the observed variations in hunger manifestation. The lymph, liver and other food reserve depots must apparently be regarded as adjuncts of the blood in this connection. Other factors may also be involved but a hunger hormone (Biedl) seems superfluous as hunger might simply reflect the metabolism of the hunger center.

Finally, the relation of food intake or the consequent central repletion to sleep might be emphasized. I found that the length of sleep depended largely upon the quantity of food ingested and the general state of nutrition. Sleep was rarely sound or refreshing after a few days of fasting. It was also shortened or made lighter by undernutrition and protein starvation. Eating late in the day tended to delay, and early eating promoted, both the natural onset and the termination of sleep. As a result of such experimentation in which a 48-hour day was used, I conclude that the eating of most of the food early in the day is contraindicated in undernourished and nervous conditions but a step in this direction seems to be a logical procedure in combating nutrogenic obesity.

SUMMARY

Evidence that hunger is determined by central rather than gastric factors was obtained by suppressing the desire to eat with an excessive food intake and finding that the subsequent gastric (hunger) contractions were then experienced as local (epigastric) sensations without hunger. On the other hand, hunger was manifested independent of gastric contractions after more or less prolonged abstinence from food. The common reference of hunger to the stomach is regarded as being largely a consequence of manifestations which are mainly incidental to hunger. When the food intake is to be restricted, an aid in hunger control is believed to be the eating of the food early in the day rather than late.

In connection with this and previous studies, I am greatly indebted to Doctor Carlson for stimulating criticism, laboratory privileges and other aid.

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BODY RIGHTING AND RELATED PHENOMENA IN THE DOMESTIC DUCK (ANAS BOSCAS)

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In a previous communication (1926) we described a number of reflexes concerned in the body righting of the domestic fowl. We found that the fowl possesses most of the reflexes described by Magnus for mammals (Körperstellung, 1924), that it lacks some of them and that it exhibits a number of body righting phenomena unobserved in mammals. These latter seem to be the result of the mode of progression peculiar to birds—flying. The flying ability of the fowl is very rudimentary indeed, and it was our intention to pass by degrees to various species of birds that are actually capable of sustained progression in the air. Before making a study of the wild duck, which is a good flyer, we first investigated the domestic duck, whose ability to fly is of about the same order as that of the fowl but which is capable of swimming and diving. It was interesting to see if aquatic birds have any special body righting reflexes as a result of locomotion on and in water.

So far as we know, the body righting reflexes of the duck have not been studied heretofore, except that Huxley (1913) and Paton (1913) in their series of investigations on the cause of apnea in the duck found that labyrinthine and cervical elements may play a part in the production of what they called postural apnea. We shall refer to their findings later on.

EXPERIMENTAL. We employed a large number of normal, unanesthetized ducks of plumage of different color and followed the same procedure that we used previously in the fowl. We shall take up the various reflexes in the same order as we did in our first paper and point out the similarities and differences between the duck and the fowl.

Static reflexes. As in the case of the fowl it is impossible to demonstrate tonic labyrinthine reflexes on the extremities in the duck. We have, however, observed a hitherto undescribed tonic reflex on the tail which we later found also in the fowl. If the duck is held by the body in the air in the supine position and the head is turned back from the posture that it assumes in virtue of the labyrinthine righting reflexes on the head, so that the neck is now stretched and continuous with the long axis of the body, or if the head and neck are dorsiflexed, the tail sharply turns downward

(dorsiflexed) and its feathers are spread. Return of the head to the normal position causes a slow straightening of the tail. This reflex is elicited through the labyrinths and not through the neck muscles, because marked dorsiflexion of the neck accompanied by twisting it in such a manner that the head is now held with its vertex up, does not cause the tail to turn down. Untwisting the dorsiflexed neck so that the vertex of the head is directed downward, immediately evokes the reflex. Moreover, in labyrinthectomized ducks this reflex is absent. Tonic reflexes elicited from the legs and exerted upon the head and tail were among the most prominent tonic reflexes we discovered in the fowl, but they are not present in the duck. Tonic neck reflexes exerted on the extremities and tail are shown by the duck fairly well. When the duck is placed in the supine position on the table and the neck is twisted so that the beak points to the right, the right leg will become extended and the tail will turn to the right. Care must be taken that the base of the neck is twisted. If that is not done, there may be no difference between the tonus of muscles of the two legs and the tail may preserve its normal position. Aside from the comparatively greater ease with which the tonic neck reflexes upon the extremities and tail can be elicited in the fowl, there is another difference between the behavior of these two species. In the fowl the wing becomes abducted on the side on which the leg is extended, but in the duck we were never able to observe any effect upon the wings. *The compensatory eye positions*, both vertical and rotatory, can be easily demonstrated in the duck.

Righting reflexes. Labyrinthine righting reflexes on the head both when the body is in the supine position and the lateral position are very conspicuous in the duck. Visual righting reflexes, i.e., the indispensability of visual impulses for the maintenance of the head in the normal position after it has been brought into that position through the labyrinths (when the body itself is in an abnormal position), are present in the duck. There is, however, a marked difference between the behavior of the duck and that of other animals in this regard. When a fowl or rabbit is held in the air in the supine position, it will bring its head into the normal position by ventroflexing the neck. If it is now blindfolded, the head will fall back, the neck will become dorsiflexed and remain in that position as long as the animal is blindfolded. Under similar conditions the blindfolded duck's head will fall back, but instead of hanging limply down indefinitely, it will be raised now and then for a short interval of time. The reason for this peculiar phenomenon will be given later, when we discuss postural apnea.

Reflexes from the body musculature on the body itself (Körperstellreflexe auf den Körper) that we could not detect in the fowl, are present in the duck. The tilting reflexes, both lateral and forward and backward, are just as pronounced in the duck as in the fowl.

Stato-kinetic reflexes. The responses to translation were the same as in

the fowl: if the duck's body is suddenly moved forward and downward the legs become extended and the toes spread. The responses to rotation are exactly the same as in the fowl and the reader is referred to our paper on the fowl (1926) for the particulars. There is, however, one response, which is very marked in the duck and which we failed to observe in the fowl. When as a result of rotation the duck's head is deviated to the right or to the left, the wing on the side toward which the head is deviated becomes abducted and remains abducted as long as the head is deviated. If the rotation is fast enough, both wings will become abducted, but in that case the wing on the side towards which the head is deviated is abducted first. This wing abduction reflex is not directly elicited by the stimulation of the semicircular canals by rotation, even though it cannot be produced by rotation of labyrinthectomized ducks. It is a secondary effect of rotation and depends directly upon the deviation of the head and neck resulting from rotation. If, while the duck is being rotated, the head and neck are held firmly in the line of the body, so that they cannot be deviated, no abduction of the wing can be observed.

Righting from the dorsal position. When the duck is placed on its back on the table it quickly turns over around the long axis of the body. Like the fowl, it executes this by a chain reflex, first turning its head, the neck and body following. If, while the duck is on its back, the neck is straightened and the head held symmetrically as regards the body (vertex downward) the duck loses the ability to turn over around the long axis of the body. It generally does return to the prone position by turning around the transverse axis of the body in throwing the back part of the body over its head and it does it much sooner than the fowl. As will be seen later this may be due to apnea produced under these conditions.

Ventroflexion of the root of the neck will result in *backward walking* in the case of the duck as it does in the case of the fowl. Sometimes the animals walk backward for a few steps only. When the duck gets its neck so twisted that it assumes its normal curvatures, it walks forward with considerable difficulty, and later when the neck again becomes ventroflexed, it walks backward. This is a new proof that progression in these two species of birds is directly dependent upon the posture of the neck.

Submergence and postural apnea. It is a well-known fact that diving animals in general are capable of holding their breath for a long time while under water. In ducks it was shown by Paul Bert (1870) that submergence for as long as sixteen minutes may not kill the animal. Later, Richet (1894) found that tying off the trachea resulted in asphyxia in 7 minutes on the average, whereas submerged ducks died after 23 minutes of apnea. It is clear that the duck makes use of a special protective mechanism in its resistance to asphyxia while under water. It was Richet also who

reported that slowing of the heart is an important factor in this increased resistance. Atropinized ducks whether asphyxiated in the air or under water died sooner than normal ones. Hastening of a fatal termination could also be obtained by sectioning the vagi. Frédericq (1893) localized the afferent nerve endings of the reflex arc concerned in the production of submergence apnea in the nostrils of the duck, since pouring water over the nostrils induced apnea. Huxley (1913) confirmed the results of these investigators but she also discovered an entirely new type of apnea in the duck, an apnea resulting from certain peculiar positions of the animal's head and neck in space. She called it "postural apnea" and she found two positions in which this type of apnea could be best elicited: *a*, the animal's body in the supine position, neck stretched or dorsiflexed, vertex of head directed downward; and *b*, long axis of the body and neck vertical, beak pointing downward. The first of these two conditions is more conducive to apnea than the second. She found that postural apnea may be elicited in lightly anesthetized and in decerebrated ducks. Huxley (1913) observed that the postural apnea was not always complete, but occasionally interrupted by respiratory movements. The longest complete apnea observed by her in normal ducks did not exceed 88 seconds, and whereas she confirmed Richet's finding that the heart was inhibited, her complete apneas were of too short a duration for the slowing of the heart to manifest itself in a very marked way.

We have repeated and confirmed the results obtained by previous investigators and have considerably extended their observations. We shall try to show why Huxley was wrong in concluding that the postural apnea was a "mechanism to aid the diving bird in resisting the aspiration of water when the head is submerged," especially as applied to the apnea produced by the dorsiflexion of the head and neck.

For our experiments we generally had the duck tied to an animal board in the supine position. The head and neck of the animal projected beyond the edge of the board so that they could be easily dorsiflexed, when that was required. A normal duck lying on its back will ventroflex its neck in such a fashion that the head is held in space in its normal position (labyrinthine righting reflex). Under these conditions the duck's beak points towards its tail. By means of a pneumograph tied around the body of the animal a respiratory tracing may be obtained. If the animal is quiet the respiratory rate is fairly uniform, varying from about 10 to 20 per minute. Thus in one duck lying quiet in the supine position the respiratory rate for successive ten minutes was as follows: 10, 13, 15, 16, 11, 13, 15, 14, 15, 16. The respiratory movements are fairly uniform in extent, but at times the respiration assumes a periodic character (fig. 1). If now the head of the animal be gently dorsiflexed, respiration continues normal until the neck has been completely straightened, the vertex directed

downward and the lower jaw horizontal. Then respiration suddenly ceases and the apnea continues for several minutes. Occasionally respiration breaks through after 4 or 5 minutes, but then the duck does not breathe more than 2 or 3 times per minute. However, we had cases of complete apnea lasting as long as 10 minutes, and incomplete apnea of 30 minutes' duration.

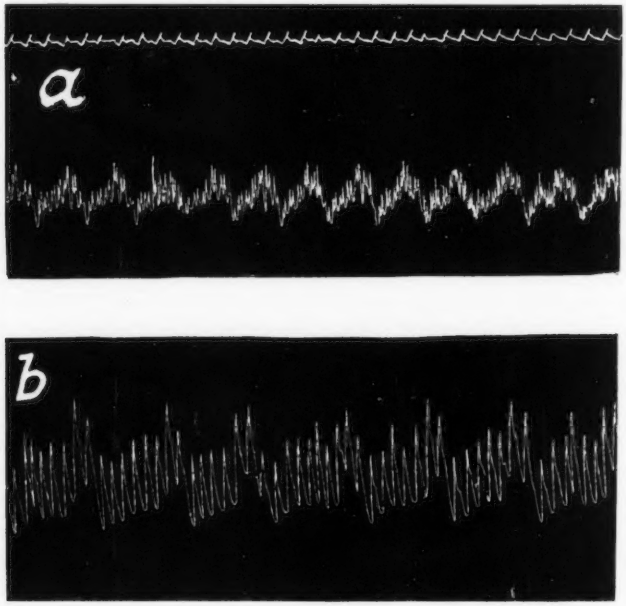

Fig. 1. The periodicity consists of a difference between the extent of the inspirations and expirations, causing alternately a gradual increase in the volume of air held in the respiratory system and a more abrupt decrease. *a*, Periodicity shown in the normal respiration of the duck; time, five seconds. *b*, Similar periodicity in respiration of another duck.

Accompanying the postural apnea there is a slowing of the heart. This was observed by Huxley in postural apnea of 15, 22 and 68 seconds' duration, and the lowest heart rate she recorded was one beat per two seconds. We used 15 seconds as a unit of time in counting the heart beats (by means of a stethoscope applied to the body wall directly over the heart) and we found the normal heart rate to vary from about 30 to 50 per 15 seconds (120–200 per minute). Under the conditions of complete postural apnea the heart rate generally fell to about 10 beats per 15 seconds (fig. 2) and

on several occasions the heart beat only once or twice or not at all in the course of 15 seconds. In one case of postural apnea 30 seconds elapsed without a single heart beat. When the head and neck are released from their "apnea" position, the heart rate, like the respiratory rate, is suddenly increased, often to such an extent that it cannot be counted, but after a few minutes it is back to normal.

A hitherto undescribed phenomenon accompanying postural apnea is a spasmodic closure of the glottis maintained throughout the period of complete apnea. This is perhaps a part of the reflex-complex that we call postural apnea. At any rate, during normal respiration the glottis is never completely closed. It is slightly more widely open during inspiration than during expiration. This spasmodic closure of the glottis in

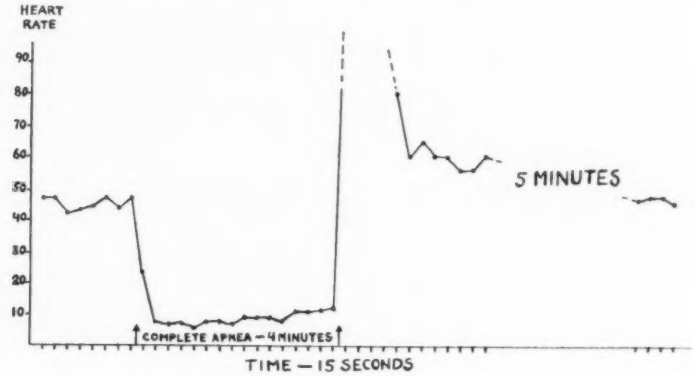

Fig. 2. The effect of complete postural apnea on the heart rate of the duck. Rate given in number of beats per 15 seconds. After the apnea the acceleration of the heart rate is so great that the heart beats cannot be counted, but it rapidly returns to normal.

apnea may be observed by direct inspection, opening the mouth of the animal and slightly elevating the tongue (the lower jaw being uppermost in the apnea position).

In four cases this postural apnea was maintained until the animals died from lack of oxygen and in several other cases the head was released and respiration allowed to be resumed at the point of death. This could be easily done, because death was preceded by generalized convulsive movements accompanied by expiratory efforts.

After a period of prolonged apnea respiratory rate was generally much higher than normal, frequently more than 35 per minute, but at the end of 10 or 15 minutes it was usually back to normal (fig. 3).

During the period of apnea the ducks would execute generalized strug-

gling movements from time to time. These movements transmitted by the pneumograph to the recording tambour could be misinterpreted as respirations. By a number of methods we succeeded in convincing ourselves that struggling involved no respiratory movements whatever and we shall now describe these methods in some detail.

A. Spirometer. By means of a special muzzle, such as described by Kunde (1923), we connected the head of the duck with the recording spirometer of the Sanborn-Benedict respiration calorimeter. Under conditions of normal respiration the writing point of the spirometer described the usual descending respiration curve, since oxygen was continually being used up by the duck. In apnea there was no movement

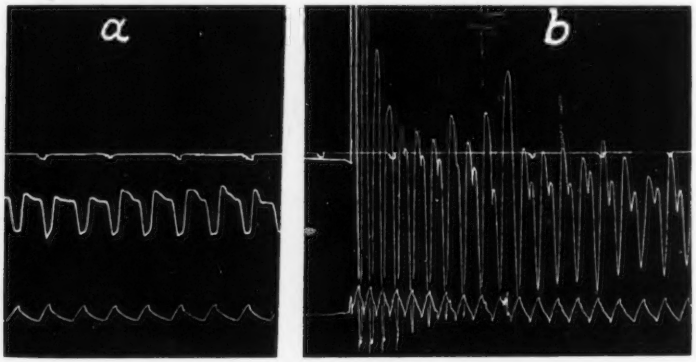

Fig. 3. *a*, Normal respiration record of the duck obtained by means of a pneumograph and a recording tambour (lowermost record); simultaneous record of the pressure within abdominal air sac obtained by means of a water manometer; uppermost tracing—time, 5 seconds. *b*, Same duck immediately after postural apnea of three minutes' duration. Respiration markedly accelerated and variations in intrasaccular pressure several times greater than normal. Gradual return to normal.

whatever of the cylinder of the spirometer, nor did any oxygen diffuse into the lungs, as the writing point recorded a horizontal line as long as ten minutes. Struggles of the animal produced no change in the horizontal line, indicating that no air was taken in or given out by the animal while it struggled. On the other hand, in incomplete apnea an occasional respiratory movement would be duly recorded on the moving paper and was always followed by the disappearance of some oxygen from the closed system.

B. Closure of the glottis. As already indicated, postural apnea in the duck is accompanied by a tight closure of the slit of the glottis. By direct inspection of the closed glottis we convinced ourselves that during

complete apnea the occasional struggles of the ducks were not accompanied by opening of the glottis, and therefore no air *could* enter or leave the trachea during these struggles. This would incidentally explain why these movements of the ducks, even though they undoubtedly involved changes in pressure of the air in the lungs and the air sacs, did not affect the cylinder of the spirometer.

C. Pressure in air sacs. The respiratory apparatus of the birds differs considerably from that found in mammals. The minor subdivisions of the bronchi do not end in blind pouches as in the mammals, but lead to the so-called air capillaries, which are lined with blood capillaries and which permit air to circulate through them in both directions. Thus in the bird theoretically at least the air does not have to go into and out of the lung—it can pass through the lung. There is an auxiliary mechanism made up of thin walled pouches called air sacs, which are connected directly with the trachea and with the lungs.[1] For the purpose of this study we were interested in the possible changes in pressure of the air in the air sacs because on a purely anatomical basis it was conceivable that even in apnea there might be a circulation of air in the lungs, and therefore oxygenation of the blood, simply as a result of alternate contraction of the abdominal and cervical air sacs.

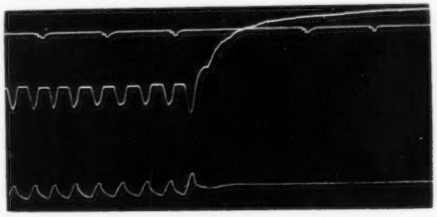

Fig. 4. Simultaneous record of respiration (lower tracing) and of the pressure within the abdominal air sac (middle tracing); upper tracing, time, five seconds. The onset of postural apnea is accompanied by a marked increase in intrasaccular pressure.

Under ether anesthesia we tied a cannula into one of the abdominal air sacs and connected it with a water manometer. After the animal recovered from the anesthesia, we made a simultaneous record of the pressure in the air sac by a water manometer and of the respiratory movements by means of a pneumograph connected with a recording tambour. We found that during inspiration the pressure of air in the abdominal sac decreased and during expiration it was increased. The fluctuations amounted to a few millimeters of water above and below atmospheric pressure (fig. 4). As postural apnea was produced the pressure in the air sac rose above that usually obtained at the end of an expiration and remained the same throughout the period of complete apnea. There could therefore be no circulation of air through the lungs during that period. If the animal struggled in the course of

[1] There are two pairs of major air sacs located in the cervical and abdominal region.

apnea, the pressure in the air sacs went up still higher, proving that no inspiration took place during the struggle.

D. Heart-rate. As indicated above, the heart rate was markedly decreased during postural apnea. If, however, the apnea was incomplete each group of respiratory movements interrupting the apnea was accompanied by an increase in the heart rate (fig. 5). Whether the slowing of the heart is a result of the apnea or whether the two are parts of one complex reflex response still remains to be elucidated, but the two always went together. The fact that the struggling movements of the animals were

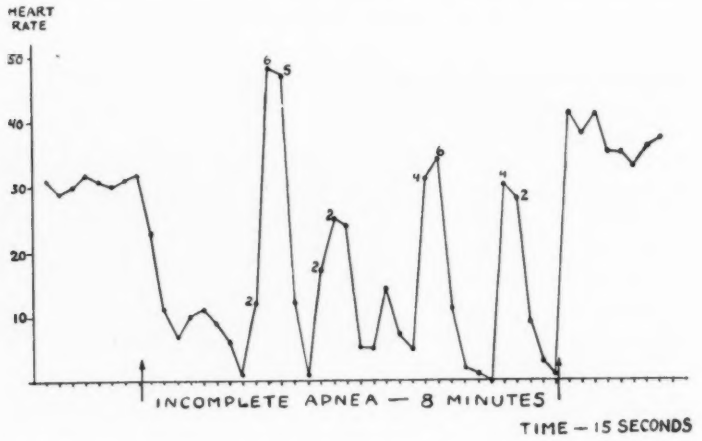

Fig. 5. The variations in heart rate during incomplete postural apnea. Rate given in number of beats per fifteen seconds. Figures accompanying plotted points stand for the number of respiratory movements executed during the corresponding fifteen second interval. It will be noted that respirations were always initiated when the heart rate fell to a very low figure, and that while the duck respired the heart was accelerated at times reaching a rate higher than normal. The increase in the heart rate after incomplete apnea is much less marked than after complete postural apnea (fig. 2).

never accompanied by increased heart rate, seems to us to indicate that there could have been no respirations during the struggles. It may be mentioned that the apnea usually was interrupted by a few respiratory movements at the time when the heart rate dropped to 2 or 1 per 15 seconds, and that the sluggish circulation with the resultant accumulation of CO_2 in the medulla might have been responsible for the overcoming of the inhibition of respiration. Apnea was generally not interrupted when the heart rate did not fall below 7 or 8 per 15 seconds (fig. 2).

We have indicated that it is possible to kill ducks by inducing and

forcibly maintaining postural apnea. But if the animal is free to move its head and neck, it will invariably bring them into normal position and thus resume breathing. This was shown strikingly by the effects of blindfolding on ducks that were held in the supine position. We already mentioned the fact that blindfolded ducks when in the supine position would let their heads fall back, but would lift them periodically for short intervals of time. It is now clear that the ducks lifted their heads to cut short the postural apnea that was induced by the dorsiflexion of the head upon the neck, and that as soon as the blood was sufficiently aerated the heads fell back again.

Huxley maintained that not all the ducks tested showed postural apnea, but that blindfolding seemed a distinct aid in the elicitation of that type of apnea. We can fully substantiate the first statement, but we are not sure that blindfolding is a deciding factor in bringing about postural apnea. We have examined a great number of ducks and we found that those that continued to breathe when in a position which in others resulted in apnea would do so whether they were blindfolded or not. We are not now in position to tell by mere inspection whether a certain duck will or will not show postural apnea.

Effects of labyrinthectomy. That stimulation of the labyrinths was one of the causes of postural apnea was very clear, but it remained to be decided whether that was the only cause. Paton (1913), on the basis of some experiments he performed, concluded that they were two afferent pathways involved in reflex postural apnea: one from the labyrinths, the other from the neck muscles. We bilaterally labyrinthectomized two ducks, which showed good postural apnea prior to the operation. These animals lost their labyrinthine righting reflexes, the tonic labyrinthine reflexes, and did not respond to translation or rotation. The tonic neck reflexes were preserved. The animals also lost their power to turn over from the supine into the prone (normal) position, and this enabled us to observe them in the supine position without the necessity of fixing them on the board. If the labyrinthectomized animal is placed on its back in such a way that its head and neck project beyond the edge of the table, it continues to breathe normally, even though the neck is dorsiflexed upon the trunk. Under these conditions the neck preserves its normal S-shaped curvature and the head does not hang listlessly downward. We found that apnea could be easily induced by dorsiflexion of the head upon the neck (occipito-atlantoid articulation). Unlike the normal duck, the labyrinthectomized duck will stop breathing as a result of the dorsiflexion of the head upon the neck in any position of the body, supine or prone. Stretching of the neck also resulted in apnea. We thus confirmed Paton in his contention that the impulses from the neck muscles play a part in the production of postural apnea, but we think that the dorsiflexion at

the occipito-atlantoid articulation is of greater importance than the cervico-dorsal articulations. We also want to emphasize the antagonism that exists between the influence of the labyrinths and the neck muscles on respiration in the normal duck under certain conditions. If in the normal animal we stretch the neck or dorsiflex the head upon the neck, we will not get apnea, if the vertex of the head is at the same time directed upward.

Submergence apnea. For the study of submergence apnea we lowered the ducks into a large, glass-walled aquarium and observed the positions which they assumed under water. At times we had pneumographs tied around the animals and connected with a recording tambour, thus obtaining a graphic record of respiration. By dipping the duck into the water, we could never detect any tendency for it to dorsiflex the head and neck. This was true whether the body of the animal was held in the prone or supine position. In the latter position, especially, with its back downward, the animal had to ventroflex its neck in order to bring the head into the normal position, and it thus deliberately changed the position of the head from one which will produce postural apnea to one which does not. We also performed the following experiment.

Holding the duck in the air in the supine position with the head and neck dorsiflexed, we produced postural apnea. In the course of this apnea we lowered the animal into the water, without changing the position of any part of the body. With the animal under water and showing apnea as a result of both posture and submergence, if the restraint upon the head is now removed, the animal will immediately ventroflex its neck and bring the head into the normal position. The apnea it now shows can thus be due only to submergence. Again, under natural conditions, when the duck dives it brings the head and neck into such a position that they are continuous with the long axis of the body, which is now vertical. If the duck is lowered into the water, when held in the diving position, the neck will immediately be dorsiflexed on the body, and the head ventroflexed upon the neck, thus bringing the head into normal position (vertex upward) and destroying any possible reinforcement of submergence apnea by the postural one. It seems, then, that postural apnea is in no way related to submergence apnea and can be no aid to the animal in diving.

In order to ascertain whether the apnea produced by pouring water over the beak of the duck is a result of the wetting of nostrils and not of water inspired with the air, we have submerged the head of the duck or the entire duck after having previously, under anesthesia, inserted a tracheal cannula and connected that cannula with a rubber tube. Even though no water could get into the trachea, under these conditions, submerging the animal or its head, or merely pouring water over its beak, always resulted in apnea.

An interesting phenomenon was observed when the ducks were allowed to emerge from the water after a prolonged period of submergence. They generally floated in such a manner that only the neck and the tail were above the surface of the water, the middle portion of the body remaining entirely submerged. The legs and feet were extended and directed backwards parallel to the long axis of the body. The tail and the hind portion of the body of the animal executed continual swaying movements from side to side, giving the impression that the animal was about to sink sidewise.

Discussion. There is very little to be said about the various static and stato-kinetic reflexes in the duck, that has not been said in our previous paper on the fowl (1926). We should perhaps emphasize some reflex responses in the duck which we did not describe in the fowl. The most important among these is the symmetrical tonic labyrinthine reflex exerted upon the tail. Since we later found the same reflex to be present in the fowl, it is probable that it is shown by birds in general. The body righting reflex from the body wall upon the body itself is present in the duck, although absent in the chicken. Finally, the wing phenomenon, which takes place during rotation and which consists of an abduction of the wing on the side to which the head is deviated, is also a response apparently peculiar to the duck. Aside from these minor differences, we could find no body righting reflexes in the duck not found in the fowl, which could be of value to the duck as a diving bird. As a matter of fact, while held under water the duck shows all the body righting phenomena that it shows in the air.

In our study of complete postural apnea we made sure, by various means, that the apnea was not interrupted by respirations during the occasional struggling movements of the ducks. Our discovery of the concomitant spasmodic closure of the glottis and the low heart rate prove beyond any doubt that no O_2 was taken in during that period. As the apnea proceeds two causes are at work in the initiation of respiratory movements: First, the absence of respiration, and second, the very sluggish circulation due to the slowed heart. Both of these are responsible for a decrease of oxygen and an increase in carbon dioxide in the tissue cells, among others in the cells of the respiratory center. The stopping of respiration being the unchanged condition, the variations in the heart rate should be the deciding factor in whether the apnea should be interrupted by respiratory movements. From figure 5 it will be seen that in incomplete apnea breathing movements are always initiated when the heart rate is at its lowest, one or two per 15 seconds. But even the bona fide respiratory movements observed in incomplete postural apnea are not sufficient to satisfy the minimum oxygen requirement of the animals. This is evidenced by the fact that some of our animals died as a result of postural apnea and others were released and allowed to breathe when at the point of death .

It would seem that the powerful excitatory action of the accumulated carbon dioxide cannot overcome the inhibitory influences on the respiratory center coming from the labyrinths and the neck muscles. This is contrary to the idea of the prepotency of the excitatory chemical stimuli to the respiratory center over the inhibitory nervous influences. The theoretical importance of this finding is obvious.

As a reason for the ability of the duck to withstand periods of prolonged apnea, the peculiarity of its respiratory apparatus suggests itself first. We have already dwelt upon the great differences between the anatomical structure of the avian respiratory system with its accessory air sacs and the comparatively simple mammalian lungs. We made a diligent study of practically the entire available literature on respiration in birds and found many contradictory statements and not one scheme of respiration based on solid experimental data. There are many attractive and plausible hypotheses, which explain how the bird *may* be breathing. (For a fine review of the literature see the recent publication of Bethe, 1927.) In such a respiratory system as exists in birds, there might conceivably exist a circulation of air through the lung from one air sac to another and back, and this would explain satisfactorily the ability of the duck to withstand apnea. However, as we have already indicated, we have direct experimental evidence that the pressure in the abdominal air sacs remains the same throughout the entire period of complete apnea.

Can postural apnea be considered as an aid to submergence apnea that follows diving, as suggested by Huxley? Whereas we have no adequate explanation for the existence of postural apnea, we feel at the same time that Huxley's hypothesis is not warranted by the facts. In the first place when the duck is placed under water it never assumes a position characteristic of postural apnea, and, in the second place, if the duck is submerged while it is held in either of the two positions which result in postural apnea, i.e., if the duck is already showing postural apnea while it is being submerged, it will immediately change its position to a normal one, if it is released from the apnea position while held under the water. Under these conditions the animal while under water has to depend for its apnea entirely upon the stimuli which usually produce submergence apnea, namely, the wetting of the mucous membrane of the nostrils (Vincent and Cameron, 1920), and makes no use whatever of the mechanism concerned in the elicitation of postural apnea.

SUMMARY

1. With the duck in the supine position, dorsiflexion of the head upon the neck (vertex downward) causes the tail to be dorsiflexed and its feathers spread. This is a symmetrical tonic labyrinthine reflex.

2. Rotation of the duck has an indirect effect upon the tonus of the

wing muscles. The wing on the side to which the head and tail are deviated during rotation becomes abducted and remains so as long as the head and neck are deviated.

3. Most of the other static and stato-kinetic reflexes observed in the fowl are also present in the duck.

4. Certain positions of the head and neck in space and relative to the position in the body will result in apnea, as already observed by Huxley.

5. Postural apnea is accompanied by persistent closure of glottis.

6. The heart is slowed and may completely stop beating for as long as thirty seconds during postural apnea.

7. If the apnea is interrupted by occasional respiratory movements there is a synchronous acceleration of the heart rate.

8. The struggling movements sometimes observed during apnea are not accompanied by actual inspiratory efforts.

9. Ducks may be killed by maintaining them in the "apnea position" for from 15 to 30 minutes.

10. Impulses from the cervical musculature and from the labyrinths are responsible for the production of postural apnea (confirming Paton).

11. From our study of the behavior of the duck while under water, we conclude that postural apnea cannot be considered as a means of reinforcing submergence apnea.

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THE INFLUENCE OF STARVATION ON THE RATE OF SECRETION OF SALIVA ELICITED BY PILOCARPINE, AND ITS BEARING ON CONDITIONED SALIVATION

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In a quantitative study of a salivary conditioned reflex in dogs (Kleitman and Crisler, 1927) it was found that the reflex deteriorated as a result of starvation and that it recovered its full strength upon realimentation. The unconditioned stimulus in this case was a subcutaneous injection of morphine, and the conditioned stimulus, a definite set of tactile and visual sensations which preceded the injection. The animals had permanent fistulae of one of the submaxillary glands. Placed in a stand, a collecting tube attached to the lower jaw, they were allowed to remain in that condition for from 15 minutes to 2 hours before 30 to 60 mgm. of morphine sulphate were injected subcutaneously. The injection called forth a secretion of saliva lasting about 20 minutes. By repeating this procedure daily for a few days a salivary conditioned reflex was gradually developed, the dogs beginning to secrete saliva *prior* to the injection of morphine, and in daily increasing quantities. If the dogs were starved while the conditioned reflex was being developed, the maximum quantity of saliva secreted conditionally was only a fraction of the quantity usually secreted under conditions of alimentation, and soon the animals would secrete less and less saliva daily, in spite of the fact that the daily injections of morphine were kept up. This abolition of the conditioned reflex could be due either to some disturbance in the central nervous system, or to a deficiency in the peripheral mechanism (the nerve endings or the secreting cells). It is well known that morphine does not stimulate the peripheral salivary mechanism, but if the latter became deficient as a result of starvation, the usual number of nerve impulses sent from the centers would result in a decreased quantity of saliva secreted. The purpose of this research was to determine to what degree the nerve centers and to what degree the peripheral structures were each responsible for the breakdown of the salivary conditioned reflex in starvation.

METHOD. As a means of testing the condition of the salivary gland injections of pilocarpine naturally suggested themselves. In a number of preliminary tests the animals were to receive a definite dose of pilocarpine,

and the quantity of saliva as well as the rate and duration of secretion determined. They could then be starved, and any difference in response to the standard dose of pilocarpine detected.

After a number of tests it was found more convenient to give the pilocarpine subcutaneously than intravenously, and a dose of 0.5 mgm. of pilocarpine sulphate per kgm. of body weight was found to be effective, yet did not make the animals sick. During starvation it did not seem right to continue giving the animals the same dose per kilo as their weight was falling, because they were undoubtedly losing more fat than active tissue, and the dose would actually be smaller. Therefore the amount of pilocarpine given on the last day of feeding was continued throughout the fast, and the dose per kilo was a little higher in starvation than during alimentation. The dogs were given injections every other day, and they were allowed to remain in the stand for 15 minutes before pilocarpine was administered. In this way one could determine, incidentally, whether the repeated injections of pilocarpine would result in the establishment of a salivary conditioned reflex.

As in the previous study the unit of time for measuring the rate of flow of saliva was 5 minutes. A record was kept of the volumes of saliva secreted in successive 5-minute periods, as well as of the total quantity collected in one hour, which was the usual length of time the animal was allowed to remain in the stand after the injection of pilocarpine. Occasionally the dogs were left in the stand for two or more hours, or until the secretion stopped altogether.

Dogs drink much less water during fasting than they do normally (Kleitman, 1927). To control the possible effect of this phenomenon on secretion, one of the dogs was given 600 to 700 cc. of water by stomach tube daily, throughout the period of starvation.

Results. The most striking result of the repeated injections of pilocarpine was the failure of all the dogs used to develop a salivary conditioned reflex. During the 15 minutes that the animals spent in the stand prior to receiving pilocarpine not one drop of saliva was secreted in the entire period of this study. Nor did the dogs vomit as a result of the doses of pilocarpine administered. They were apparently not nauseated, because they would eat heartily after being taken out of the stand. The significance of this will be discussed later. It should be pointed out now that for the purpose of this investigation absence of conditioned secretion was very favorable, since it meant that all the saliva obtained as a result of the pilocarpine injections was due to its action on the peripheral mechanism only.

As regards the total quantity of saliva collected in one hour after the injection of pilocarpine it may be said that it was not uniform for any of the dogs studied. The injections were made subcutaneously, and certain

TABLE 1

Volumes of saliva (in cc.) collected in one hour from salivary fistulae of three dogs during alimentation, starvation and realimentation

DOG H	DOG T	DOG L	REMARKS
28.4	53.3	45.1	Period of alimentation
22.5	46.3	49.2	
23.0	54.6	40.7	
33.8	44.8	49.1	
Average 26.9	49.8	46.0	
32.7	36.9	26.9	Period of starvation
18.2	28.8	36.5	
22.2	62.5	31.1	
17.8	42.4	36.2	
19.3	45.2	35.5	
20.9	43.2	22.2	
17.4	48.3	41.0	
15.7	42.6	39.3	
13.6	34.5	42.0	
15.0	29.1	28.5	
13.2	26.8	31.5	
9.7		34.2	
Average 18.0	40.0	33.8	
21.6		39.3	Period of realimentation
24.3		41.1	
28.3		53.0	
22.1		43.6	
20.2		40.9	
18.0		47.6	
21.3		50.7	
15.8		38.2	
13.8		37.8	
11.2		38.7	
21.8		37.5	
24.8		41.4	
20.9		41.2	
19.1		37.9	
22.6		38.3	
24.5		39.7	
16.5		41.0	
26.8		44.3	
24.8		41.5	
24.9		44.2	
Average 23.2		41.7	

fluctuations could be expected from the differences in the rate of absorption of the drug into the circulation. Thus in dog L, weighing about 15 kilos, the quantities of saliva collected in one hour during alimen-

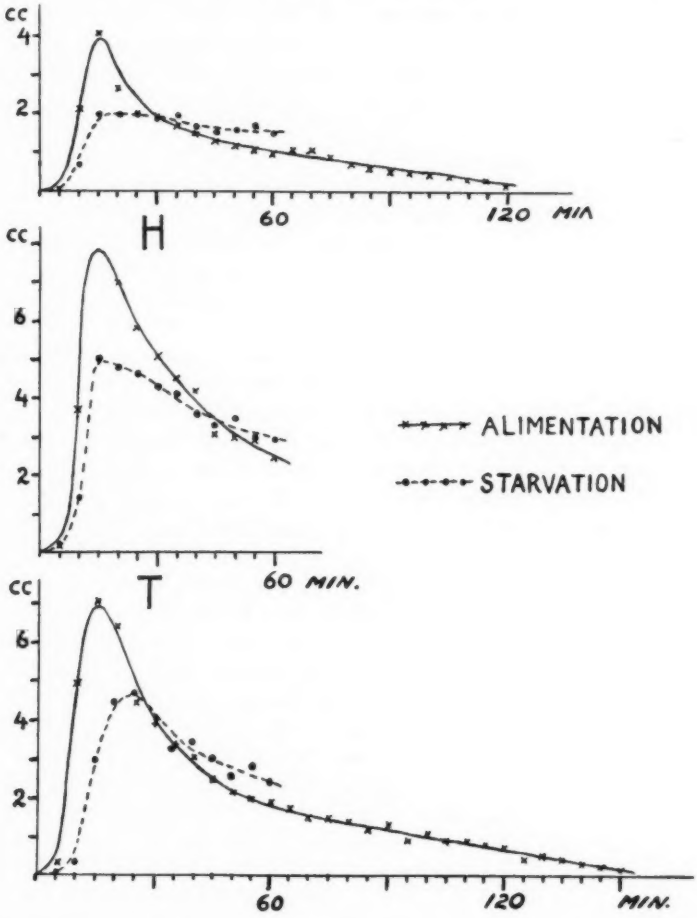

Fig. 1. The average secretion rate for successive 5-minute periods of dogs, H, T, and L during alimentation and in starvation. The portions of the curves for H and L that extend beyond the first hour are based on a smaller number of observations than the rest of the curves.

tation varied from 37.5 cc. to 53 cc., the average being 42.6 cc. and the average deviation only 3.5 cc. These values obtained during periods of

alimentation preceding and following a period of starvation furnish a standard with which to compare the total quantities of saliva secreted in starvation. From table 1 it will be seen that there was just as great a variation in the total volume of saliva secreted in one hour in starvation as there was during alimentation. In dog L there was no consistent gradual decrease in the volume of saliva in the 24 days of starvation. Dog T began to show such a decrease after 16 days of starvation (the dog began to look unwell at that time, and the experiment was discontinued after 22 days of starvation so far as T was concerned). Dog H was the only one that showed a definite tendency to secrete less with the progression of the starvation process. However, all dogs show a marked decrease in the total volume of saliva secreted during starvation, this decrease amounting to from 20 to 33 per cent of the normal. In dogs L and H there was an immediate partial recovery of the secretory activity of the salivary glands upon realimentation, but the recovery was not complete after 40 days of realimentation.

A significant difference can also be detected in the rate of secretion of saliva during alimentation and in starvation. As a rule no saliva was secreted in the first five minutes after the injection. Then the secretion rate rapidly reached a maximum and thereafter gradually fell. The maximum was attained in the second, third or fourth 5-minute period, and, as the composite curves of the secretion rate (fig. 1) show, during alimentation the average maximum was in all cases in the third 5-minute period. After the third period the secretion rate drops, first more abruptly and later more gradually, until at the end of two hours or so it stops completely. The curve for the secretion rate during starvation differs in several ways from the normal curve. The height of secretion is reached between the third and the fifth 5-minute periods after the injection and is on the average much lower than the normal maximum. That is also reflected in the figures for individual 5-minute intervals. Thus dog H, in 24 days of starvation, secreted more than 4 cc. in 5 minutes only on two occasions, whereas during 40 days of realimentation he did that twelve times. The most interesting feature of the composite curves of secretion during starvation is the much more gradual decline after the peak had been reached. Dog T that received water by stomach tube does not show a secretion curve for the period of starvation differing in any way from those for the other two dogs that were allowed to drink as little water as they pleased.

Discussion. Several conclusions may be drawn from the rather limited data presented above. The first significant fact revealed is the failure of the dogs to develop a conditioned salivary reflex as a result of repeated injections of pilocarpine. A conditioned reflex is generally developed by repeated simultaneous application of the usual (or unconditioned) stimulus

to some sensory nerve terminations and of an unrelated (or conditioned) stimulus to some other receptor organ. In this sense the morphine-salivary conditioned reflex is not in strict conformity with the above definition, since the injection of morphine, the unconditioned stimulus, does not act on any definite sensory end-organ, but on a center or centers in the nervous system, getting there by a humoral route. However, the conditioned stimulation, which consists of a variety of tactile and visual sensations attendant upon the dog's staying in the stand, is capable, after a period of training, of eliciting, among other phenomena, a reflex flow of saliva. That means that a conditioned response may be developed in an animal by associating the conditioned stimulus with an excitation of either the afferent end or the center of the "unconditioned" reflex arc. The experiments with pilocarpine, which presumably acts on the junction between the efferent nerve endings and the cells of the salivary glands, indicate that it is impossible to develop a conditioned salivary reflex in the dog when the unconditioned stimulus is applied to the efferent end of the reflex arc.

In starvation the volume of saliva obtained after an injection of a constant dose of pilocarpine is much smaller than during alimentation. That is probably partly due to the gradual decrease in the size of the gland. That there are other influences is shown by the fact that the average decrease in the volume of saliva secreted during starvation was 33 per cent for dog H, 30 per cent for dog L, and only 20 per cent for dog T. Dog T, it will be recalled, received water by stomach tube daily. Thus the smaller water intake during starvation (Kleitman, 1927), while it has an influence on salivary secretion, will account only for about 10 to 15 per cent decrease from the normal. But whatever the causes of the decrease in salivary secretion in response to pilocarpine, and marked though this decrease be, it cannot be compared with the practical abolition of the conditioned reflex secretion due to morphine which we observe in starvation. The original question which occasioned this research was: can the deterioration of the conditioned reflex during starvation be brought about by changes in the periphery alone? This question must now be answered in the negative. We are led to the conclusion that during starvation the physiological condition of the central nervous system itself is changed to such an extent that conditioned reflexes, or at least the particular salivary reflex studied, cannot be developed fully or, if already developed, cannot be maintained, in spite of the continued application of the unconditioned with the conditioned stimuli.

Incidentally the rate of secretion of saliva in successive 5-minute intervals was studied. The action of pilocarpine on the salivary apparatus being a purely peripheral one, we feel justified in assuming that the quantity secreted in a unit of time is the function of the concentration of the drug

in the blood. Possible fatigue of the nerve-endings as a cause of the gradual decrease in secretion must be excluded, because in our study of the morphine-salivary conditioned reflex dogs secreted saliva by central stimulation for two or three hours at a higher rate than they ever secreted after an injection of pilocarpine. The curves in figure 1 show that with the doses used pilocarpine reaches its greatest concentration in the blood, on the average, about 15 minutes after the subcutaneous injection and that it is excreted for the next 15 minutes rather fast, then more slowly, and that traces of it remain in the blood as long as two and a half hours after the injection. During starvation absorption into the blood stream is somewhat slower than normal, but elimination is considerably slower. During the last three 5-minute periods of the first hour after the injection each of the dogs secreted more saliva during starvation than during alimentation, showing that pilocarpine lingered in the blood longer in starvation.

SUMMARY

1. Repeated subcutaneous injections of pilocarpine do not result in the development of a salivary conditioned reflex in the dog.

2. There is a decrease in the volume of salivary secretion elicited by a uniform dose of pilocarpine as a result of starvation, due partly to an atrophy of the salivary glands, partly to a diminished water intake.

3. The practical abolition of the morphine-salivary conditioned reflex in starvation is probably the result of a physiological deficiency in the central nervous system.

4. On the basis of the salivary secretion rate, pilocarpine, when given subcutaneously, is absorbed slower and eliminated much slower in starvation than during alimentation.

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THE EFFECT OF REFLEX EXCITATION AND INHIBITION ON THE RESPONSE OF A MUSCLE TO STIMULATION THROUGH ITS MOTOR NERVE

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Although some of the salient properties of functional activity in nerve and muscle have been revealed by recent researches, the significance of these properties in connection with the physiology of the central nervous system is still largely unrecognized. In the older literature we find the nervous transmission of excitation spoken of as if it were a continuous stream whose intensity could be graded by varying the strength of stimulation, much as a stream of water can be regulated by opening or closing a faucet. Little attention was paid to the individual nerve impulses which composed the stream. From a large body of investigations (Lucas, 1912, 1917; Adrian, 1914, 1924; Pratt, 1917; Kato, 1924; Davis, Forbes, Brunswick and Hopkins, 1926) following chiefly from the impetus of Lucas' work, it has become well established that the functional responses in nerve and muscle are fundamentally alike. In either case there is a transient disturbance, dynamically resembling an explosion in that the energy is derived from the active tissue and not from the source of stimulation. The response is followed by a refractory period during which the tissue is irresponsive to stimulation. From these facts, as well as from direct observation, it became evident that the response is essentially of an all-or-none character, and not subject to qualitative variation. Yet theories and hypotheses are from time to time advanced, which are quite incompatible with these known properties of nerve and muscle response. One still sees occasionally in the literature a tendency to ignore the implications of the all-or-none law and to assume that the size of the nerve impulse can be graded by varying the strength of stimulation. One also finds some writers (Langelaan, 1915; Hunter, 1924) who in order to explain the phenomena of tonus, postulate a type of contraction qualitatively different from that evoked by direct stimulation. Weiss (1924, 1926) has endeavored to explain reflex coördination on the basis of the tuning of muscles to special types of excitation in the nerve, on a principle either involving or resembling resonance. The incompatibility of this theory with the known properties of nerve and muscle has already been

emphasized (Detwiler, 1925; Forbes, 1926). Weiss contends that the all-or-none principle applies only to artificial excitation, and not to the activity of nerve arising in the natural way from spinal centers.

A tissue which responds to excitation with an all-or-nothing release of energy from an unstable system, can hardly be supposed to respond in any other way, whether the source of excitation is artificial or natural. Moreover, there is experimental evidence from several sources tending to show that the nerve impulse arising from its natural source is essentially the same as that evoked by artificial stimulation. Thus Forbes and Gregg (1915) recorded a diphasic action current in the motor nerve in response to reflex stimulation, which exhibited essentially the same time relations as that evoked by a single induction shock applied directly to the nerve. Forbes, Campbell and Williams (1924) recorded asynchronous discharges of proprioceptive impulses in an afferent nerve. They found that not only did action currents appear, as in the case of artificially induced impulses, but when the mode of leading off the action currents is changed from diphasic to monophasic the picture undergoes precisely the change that would be expected on the assumption that artificial and natural impulses in the individual fibers are the same. Adrian and Zotterman (1926) have directly demonstrated the all-or-none character of the action currents of impulses arising from intra-muscular receptors, and Adrian (1926) has drawn attention to the essential similarity of these disturbances to those of touch and pain. Decerebrate rigidity, which some authors have considered a fundamentally different type of contraction from that involved in voluntary motion, presents substantially the same type of action current, and most of the available evidence leads us to suppose that the rigidity is produced by essentially the same type of activity, namely, an appropriate temporal sequence of all-or-none responses of individual muscle fibers. Such evidence tends to show that natural responses of nerve and muscle are marked by action currents indistinguishable from those which mark responses artificially induced, and since the action current is the most direct indication of functional response, there is a strong presumption that the activity is essentially the same, however produced. Since the above evidence is somewhat fragmentary, any further data tending to show the essential identity of response to artificial stimulation with that of central origin, is worth recording.

Inhibition may be central or peripheral. Such tissues as the heart and smooth muscle, which exhibit spontaneous activity, are subject to peripheral inhibition. But such a mechanism is superfluous in the case of skeletal muscle, for as Sherrington pointed out (1913, p. 253) contraction of skeletal muscle normally results only from a discharge of impulses from the central nervous system; the cessation of motor nerve impulses suffices for the complete cessation of muscular contraction. Verworn (1900) showed that reflex inhibition of skeletal muscle is in fact central and not

peripheral, for such inhibition does not reduce the size of contraction which a motor nerve stimulus evokes in the muscle.

In view of the all-or-none character of the nerve impulse it is impossible by further stimulation to add to the activity of a fiber already occupied. Evidently, then, if the central discharge consists of impulses of the same character as those evoked by artificial stimulation, it must, while in progress, render the fibers involved refractory to further stimulation; consequently we should expect a central discharge of nerve impulses to prevent the innervated muscle from responding to stimulation of its motor nerve. If all the motor nerve fibers are at the moment busy conducting impulses from the center, a stimulus applied to the nerve should evoke no additional contraction; if none are busy, the motor-nerve stimulus should evoke a maximal contraction. The size of the muscle response to such motor-nerve stimulation should be an approximate measure of the number of nerve fibers not occupied at the moment; it should be greatest during complete central inhibition.

In a preliminary communication (Whitaker and Forbes, 1921) on the earlier experiments of our series, the use of submaximal stimuli applied to the motor nerve was mentioned. Such stimuli are those which excite some fibers in a resting nerve and fail to excite others. The reason for using submaximal stimuli was that, during the relative refractory period following each response in the nerve fiber, a strong stimulus may excite when a weak stimulus will fail. We have no evidence that in normal activity the component neurones of a motor nerve discharge impulses in synchronous volleys (see Forbes and Barbeau, 1927). Presumably the individual fibers conduct impulses in an irregular sequence, usually out of phase with their neighbors. If this is so, then at any given moment some fibers will be in the act of responding and therefore in the brief absolute refractory phase, some in the less brief relative refractory phase, while others may be completely recovered from the refractory phase and will have a normal threshold. Very strong stimuli would excite not only the recovered fibers, but those in the relative refractory phase, whereas weaker stimuli would excite only those fibers which have almost or completely recovered. Therefore if each fiber is for a large percentage of the time in the relative refractory phase, submaximal stimuli should differentiate more effectually than stronger stimuli between a busy and an idle nerve.

Since the preliminary report was published the experiments have been extended to include supramaximal[1] as well as submaximal stimuli, in order that a comparison could be made between the two.

[1] By supramaximal we mean any stimulus greater than that which just suffices to evoke a maximal response. This meaning is distinct from that which has been applied to repetitive responses from a strong single stimulus (cf. Fulton, 1926, p. 409).

METHOD. In all experiments decerebrate cats were used. Transection of the brain-stem at the level of the anterior colliculi was carried out under deep ether anesthesia by means of the Sherrington guillotine. The ankle extensor muscle—gastrocnemius—was used as an indicator; stimulating electrodes were applied to its uncut motor nerve (popliteal). To this end the peroneal and popliteal branches of the sciatic nerve were carefully dissected apart with a sharp scalpel. The stimulating electrodes were applied by means of the Sherrington glass shield with a slot cut in the side to permit application to the nerve without cutting it. The shield was secured in position as firmly as possible with sutures passed through the surrounding muscles. Throughout the operation the nerve was handled with care to avoid stretching it or jamming it against the glass at either end of the shield.

In absence of afferent stimulation, the gastrocnemius muscle, an ankle extensor, remains in decerebrate rigidity. This tonic reflex contraction can be inhibited by excitation of any afferent nerve in the hind limb (Sherrington, 1906). To induce the reflex inhibition which was to be contrasted with reflex excitation, stimulating electrodes were applied to the central portion of the peroneal nerve severed at the knee. In the earlier experiments the crossed extension reflex was induced by pinching the toes of the opposite leg. In the later experiments stimulating electrodes were applied to the sciatic nerve of the opposite leg. Stimuli could be delivered to either the excitatory or the inhibitory afferent nerve at frequencies up to several hundred a second by an inductorium and a rotary interrupter (Forbes, 1921; Querido, 1924). In most of our experiments the frequency was about 60 break shocks to the second.[2] A second inductorium with a mercury-copper key provided single make and break shocks to stimulate the motor nerve to the gastrocnemius. Movement of the foot due to contraction of the gastrocnemius muscle was recorded on a smoked drum by means of a light aluminum lever, whose motion was slightly restrained by an elastic band, in order to diminish overshoot.

As a rule action currents of the muscle were also recorded. A Cambridge string galvanometer was used in the earlier experiments, and later a Hindle instrument with a 1.5 mm. air gap. The galvanometer was connected to the muscle by means of agar and silver chloride electrodes (see Forbes and Olmsted, 1925, p. 25), whose wicks were secured to the surface of the muscle by sutures sewed through the fascia, small "windows" being cut in the skin to provide access. The femur and the tibia were rigidly clamped, and the hamstring nerve was severed to prevent mechani-

[2] In most experiments the make shocks as well as the break shocks were effective and, therefore, the stimulus frequency was double the above figure.

cal disturbance due to flexion of the knee. The arrangement is shown diagrammatically in figure 1.

The threshold and maximal values of the motor-nerve stimulus were first determined. The usual procedure was *a*, to stimulate the motor nerve three or four times with break shocks of constant strength (submaximal or supramaximal as the case might be); *b*, to apply tetanic stimulation to the opposite sciatic nerve, thus inducing the crossed-extension reflex, and while this was in progress to stimulate the motor nerve again

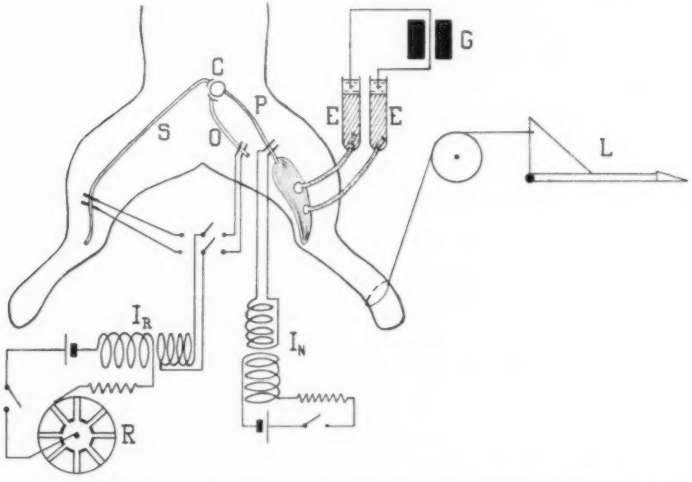

Fig. 1. Diagram of arrangement of preparation and apparatus. I_R, inductorium for evoking reflex excitation or inhibition. *R*, rotary interrupter for reflex stimulation. I_N, inductorium, for stimulating motor nerve with single shocks. *S*, sciatic nerve used to evoke crossed extension reflex. *O*, peroneal nerve used to inhibit the reflex. *P*, popliteal, motor nerve to gastrocnemius muscle. *C*, spinal center. *E*, *E*, nonpolarizable leads connecting muscle with string galvanometer *G*. *L*, muscle lever for recording on kymograph.

three or four times; *c*, shifting the tetanic stimulation to the ipselateral nerve, so that reflex inhibition ensued, to stimulate the motor nerve three or four times more during inhibition, and finally *d*, to withdraw the reflex stimulation altogether and stimulate again three or four times while the reflex activity of the muscle was returning to its normal state of decerebrate rigidity, or even to that heightened state known as "rebound" (Sherrington, 1908). In some cases the excitatory reflex stimulation was omitted; in these cases the responses during reflex inhibition were compared with those observed in the preceding decerebrate rigidity and in

subsequent rebound, when this occurred. In some cases in which we found no decerebrate tonus, the inhibitory stimulation was omitted and reflex excitation was simply compared with the preëxisting inactivity.

Uncertainty as to whether the writing lever might have enough tendency to overshoot to confuse the mechanical results led us to repeat the experiment with the isometric lever set up by one of us for other researches (Pi-Suñer and Fulton, 1927). With this apparatus a Cambridge string galvanometer was used and the results, both electrical and mechanical, were recorded together by means of a falling-plate camera. In these experiments all muscles of the hind limbs except those under observation were paralyzed by nerve or tendon section in order to prevent mechanical disturbance.

RESULTS. The experiment with the isotonic lever was performed on forty-two animals, but only in a minority of these were definite and clear-cut results obtained, for the experimental difficulties proved great and were not always successfully overcome.

In the first place it was essential to the success of the experiment that the motor nerve should be free from damage. In view of the great susceptibility of mammalian nerves to injury by tension (see Forbes and Ray, 1923), it was somewhat difficult to avoid significant trauma. In one experiment in which the hind-limb muscles were not denervated, the gastrocnemius muscle, though failing to respond to reflex stimulation, contracted when stimuli were applied directly to the motor nerve at the hip, yet reflexes were observed in all the other limb muscles. The inference was that the handling to which the nerve had been subjected established therein a partial block, that the full-sized impulses set up by direct stimulation were able to pass the block, but that the reflex motor-nerve impulses were largely subnormal by virtue of their high frequency, and therefore unable to pass, (cf. Forbes and Olmsted, 1925).

Decerebrate rigidity may be for a long time inhibited, if persistent afferent nerve activity is set up by operative procedures. Such a condition will in large measure defeat the aim of the experiment.

Sherrington and Sowton (1911) have shown that the afferent stimulus which normally causes reflex inhibition may also in some cases induce reflex excitation of the extensors in the stimulated limb. There appears to be an excitatory content in the reflex effect of such stimulation, normally masked by a dominant inhibitory content (cf. Forbes, 1921). In some cases the excitatory content is dominant and it is difficult to obtain anything approaching complete inhibition of the extensor muscle (Fulton and Liddell, 1925). This condition, when present, also defeats the object of the experiment.

It is difficult to place the stimulating electrodes in such a way that their contact with the motor nerve will not shift as the muscles of the limb contract or relax. A submaximal stimulus is one which does not excite all of the fibers in the nerve; therefore a change in electrode contact is almost certain to change the percentage of fibers which a given induction shock will excite. Therefore, if electrode contact is variable, all records made with submaximal stimuli are rendered too irregular and uncertain to be of value, and in those early experiments in which we used submaximal

stimuli only, the results of a whole experiment were occasionally invalidated through this cause. In some of the earlier experiments we were fortunate in avoiding these difficulties and obtaining fairly uniform results. In the later experiments we learned to use more dependable methods and to arrange experimental conditions in which a large number of observations with consistent and fairly uniform results could be obtained.

In the case of submaximal stimuli we should expect a certain small percentage of exceptions to the general rule which would follow our working hypothesis. If in reflex activity the impulses are distributed at any given moment in a haphazard way among the individual fibers of the motor nerve, the probability would be that the greater the proportion of motor fibers which were active, the fewer would there be free to respond to the direct stimulus; but occasionally it might happen that the motor nerve stimulus would excite a considerable group of idle fibers at the time when a large group of remaining fibers, to which the stimulus was subminimal, was conducting a volley of impulses to the muscle. The result would be a larger response in the muscle than if the same stimulus came at a time of complete inactivity.

The interpretation of the myograph records on the smoked drum also presents a certain difficulty. Here the criterion for estimating the size of a muscle response to motor-nerve stimulation is the height of the excursion marking the neuromyal twitch; but since we are comparing contractions induced during reflex activity with those induced during reflex inhibition, these excursions start from a different base line in the two cases. It becomes a question, then, whether to judge the size of response by the amount of excursion from the base line from which it starts, or by the absolute level which it reaches. In some cases the change of base line was small, and the increased size of response during inhibition was so great that the muscle lever not only made a larger excursion from this base line, but reached a higher absolute level during inhibition than during the preëxisting tonus, or even during reflex excitation. The inference in such cases was obvious; but in other cases reflex inhibition caused a large fall in the base line and the excursions due to motor-nerve stimulation, though larger than those during reflex excitation, still failed to reach so high a level. In such cases it was impossible to judge with certainty whether the mechanical responses during reflex inhibition should be considered larger or smaller. In other cases the difference in base line was so great that the possible difference in overshoot of the lever introduced uncertainty. A criterion which eliminates these sources of confusion is the action current.

Let us consider first the earlier experiments in which only submaximal stimuli were applied to the motor nerve. In these there was a marked preponderance of observations in which the response to motor-nerve stimulation was smaller during decerebrate tonus, rebound contraction and the crossed extension reflex, than during reflex inhibition. In general the size of response seemed to be inversely correlated with the degree of preëxisting reflex activity, whichever of the three types it was. Seven experiments, in which conditions were stable enough to warrant generalization, all showed a majority of observations in which the electric responses conformed to the above rule. This majority varied, in the different experiments, from 65 per cent to 85 per cent of all the observations. In arriving at these figures each observation is taken to consist in a series of

from 5 to 15 successive responses in which the background of reflex activity was changed back and forth between excitation and inhibition, so that several responses in each condition could be averaged. The remaining minority consisted of observations in which there was no consistent difference, or in which the average response during inhibition was actually smaller than that during the decerebrate tonus or reflex excitation which preceded and followed inhibition. It has already been noted that with

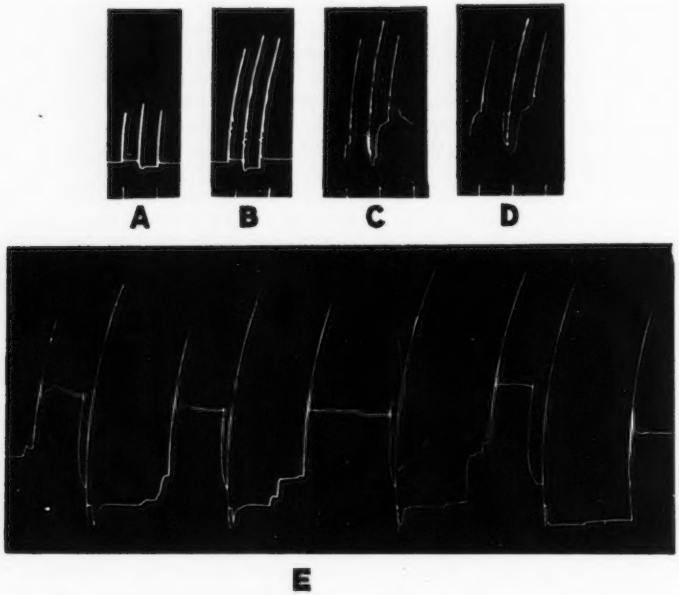

Fig. 2. Myograph records showing increased size of muscle twitch in response to motor-nerve stimulus during reflex inhibition, as compared with that during reflex activity (see text).

A, *B*, *C* and *D*, June 10, 1920. Speed of drum shown by 10-second intervals below myograph. Motor-nerve stimulus in Z units—*A*, 28; *B*, 31; *C*, 38; *D*, 48; threshold, 24. *E*, July 16, 1920, drum moving continuously throughout this observation. Motor-nerve stimulus in Z units 35; threshold, 31.

submaximal stimuli a minority of exceptions to the rule must be expected when the stimulus excites a group of idle nerve fibers at the same moment that another group is responding reflexly.

In figure 2 are shown several examples of myograph tracings in which the twitch evoked during reflex inhibition actually raised the writing lever to a higher level than those which preceded and followed it during decerebrate rigidity, crossed extension reflex or rebound. In these cases the mechanical

record leaves very little doubt that the responses during inhibition were the larger. The first two observations shown in the figure compare inhibition with decerebrate tonus, which in this case was slight. In the next two observations (from the same experiment) the crossed extension reflex was induced by pinching the toe of the opposite foot, both before and after inhibition, in order to establish a better background of reflex activity than was afforded by decerebrate tonus. The same procedure was used in the remaining observation of this figure (July 16).

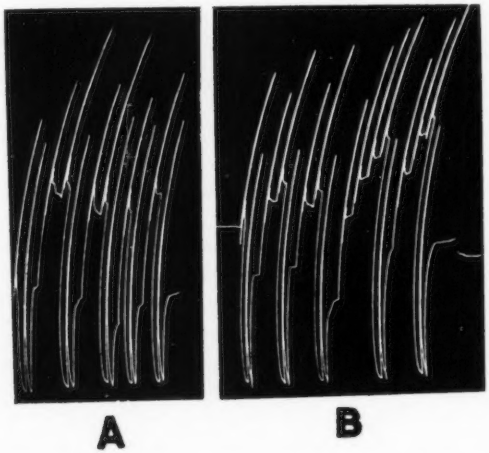

Fig. 3. Two groups of submaximal responses from the experiment of September 3, 1920. In each group the first motor-nerve response was evoked during decerebrate rigidity, which was strong; the second response and all others rising from the lowest level were evoked during complete reflex inhibition, the others during rebound which regularly followed inhibition. The drum moved continuously while each group of responses was recorded; the speed can be determined from figure 4, in which portions of each group are redrawn to a different time scale. Motor nerve stimuli, 123 Z units.

In many of our observations the mechanical records were equivocal because, as stated above, the absolute level attained fell with the base line. Examples of such records are shown in figure 3.

The electromyograms eliminate this source of confusion. Figure 4 shows a series of such records from the experiment in which the characteristic change is revealed most strikingly and consistently. These records are portions of the electromyograms taken simultaneously with the mechanical records in figure 3. Other similar records were made in many experiments, but in none did the reducing effect of preëxisting reflex activity

appear quite so marked as in this one. In this figure the action-current records are shown in pairs, each pair contrasting a response evoked during inhibition with one during reflex activity. Clearly the response was much smaller when the muscle was already engaged in reflex activity.

In these electromyograms decerebrate tonus and rebound appear indistinguishable. The irregular series of action currents is similar, and in each case there is a decrease in size of response to motor-nerve stimulation, corresponding roughly with the apparent degree of activity at the time the stimulus was applied. It is interesting to note that during decerebrate tonus and rebound the response evoked by the motor-nerve stimulus is followed, after an interval of about 70σ, by a second well-defined action current in the muscle, whereas little or no evidence of such an occurrence can be seen in the responses evoked during inhibition. The inference is that a proprioceptive reflex or ankle jerk resulted from the artificially induced twitch of the muscle, and that when the reflex inhibitory stimulus was applied this reflex was inhibited together with the decerebrate tonus.

All records thus far considered were made with submaximal stimulation of the motor nerve. In the introduction we stated that submaximal stimuli were chosen as being likely to differentiate more sharply than supramaximal stimuli between busy and idle motor nerve fibers. Our experience in these early experiments led us to the conclusion that in spite of this advantage submaximal stimuli offered a less reliable method of research than supramaximal stimuli. There are two distinct reasons for this; one is the possibility of shift of contact, which has already been mentioned. If the stimulating electrodes are not well secured in place, irregularity of response to submaximal stimuli will probably reveal the shift of contact, but even if the electrodes are firmly secured there may be a slight change of contact recurring regularly every time the muscle contracts. Such a change may alter the percentage of fibers excited and thus introduce a constant error, but the error will not reveal itself by irregularity in the contractions. This source of error may be avoided by using supramaximal stimuli, which can be relied on to excite all the fibers in the nerve as long

Fig. 4. Electromyograms showing increased size of electric response of muscle to motor-nerve stimulus during inhibition, as compared with that during reflex activity. The action currents were recorded simultaneously with the records shown in figure 3, as follows:—*A1*, corresponds with the 7th and 8th contractions shown in figure 3A, *A2* with the 10th and 11th. *B1* corresponds with the 16th and 17th contractions in figure 3B, *B2* with the 2nd and 3rd in figure 3B.

Above each electromyogram is shown a rough sketch of the mechanical changes shown in the corresponding portions of figure 3, drawn to time scale of electromyogram. Time shown by tuning fork shadow; 1 d.v. = 0.01 second. Cambridge galvanometer; 5-μ, 900-ohm platinum string; magnification, 300; string tension, 45 mm. per amp. (see Forbes and Ray, 1923).

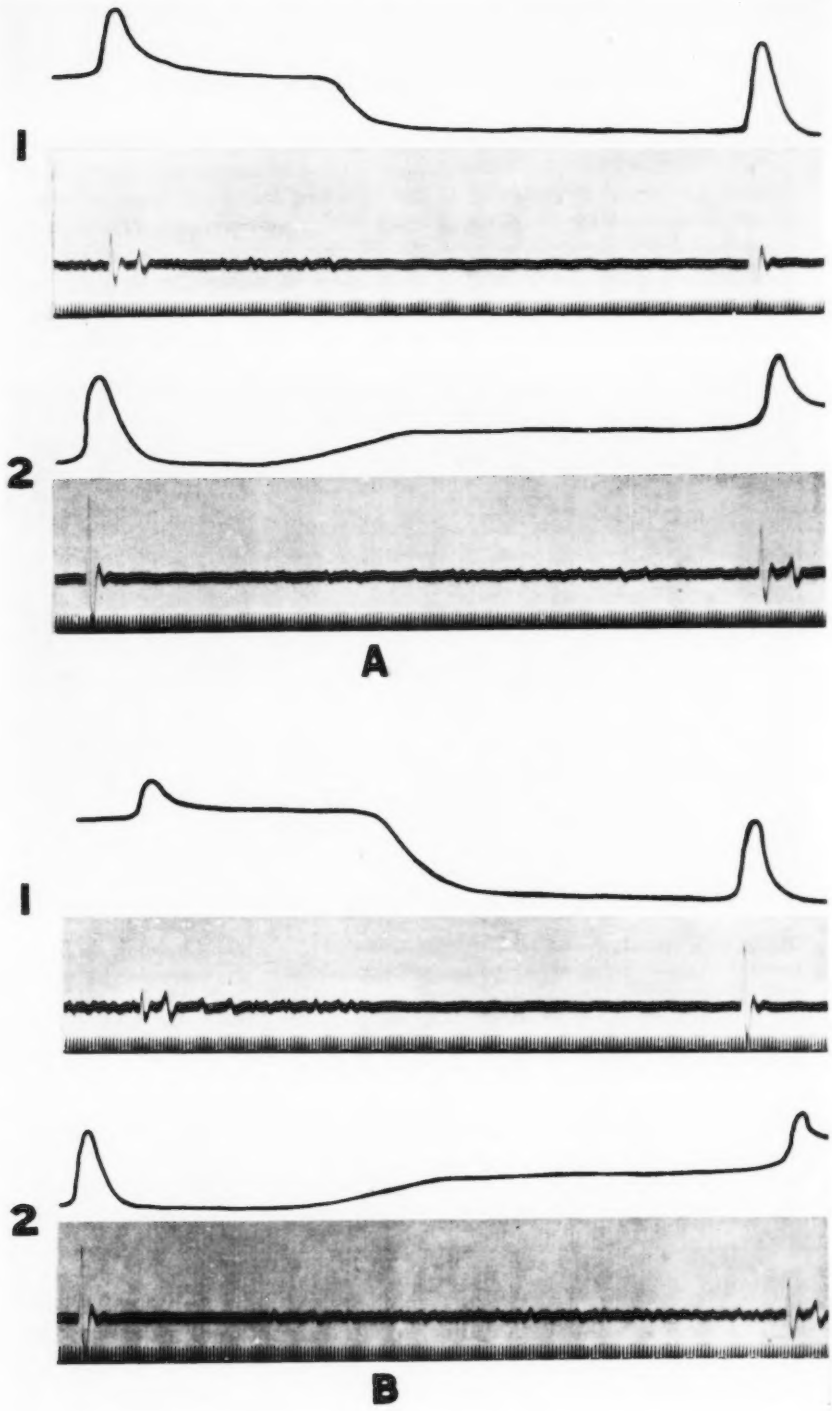

Fig. 4

as electrode contact is maintained. The other reason for the greater reliability of supramaximal stimuli is the fact, also mentioned above, that statistically a certain minority of exceptions to the general rule is to be expected with submaximal stimuli when the fibers most accessible to the stimulus are excited at the moment of a reflex discharge in a considerable group of less accessible fibers. Supramaximal stimuli, by exciting all idle fibers, should eliminate the possibility of these exceptional results. For these reasons in our later experiments we used supramaximal stimuli; we

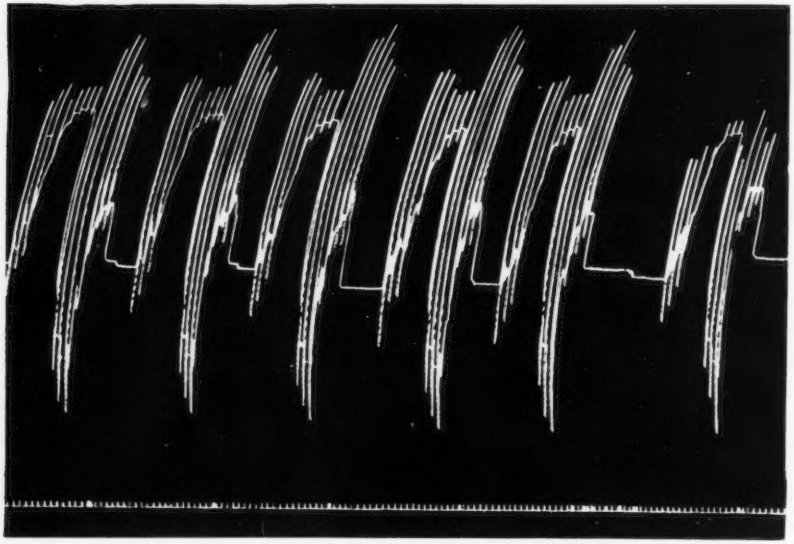

Fig. 5. Responses to motor-nerve stimuli in groups during decerebrate rigidity, crossed extension reflex, inhibition and rebound, successively. November 26, 1924. In each group the drum was moving continuously and the strength of the motor-nerve stimuli remained constant. Between groups the drum was stopped and the strength of stimulus was altered. 1st group, 17 Z; 2nd, 14 Z; 3rd, 11 Z; 4th, 9.4 Z; 5th, 8 Z; 6th, 6Z. Time shown in seconds below.

also from time to time applied submaximal stimuli, for purposes of comparison.

As in the case of submaximal, so with supramaximal stimuli, the mechanical records sometimes show clearly the effect of pre-occupation by reflex activity in the motor nerve.

Figure 5, from our most complete and thoroughly controlled experiment, shows a series of observations in which the strength of stimulus applied to the motor nerve was progressively altered from well above

maximal to a submaximal value not much above threshold. In each observation of this series the standard procedure was regularly employed; that is, the first three break shocks were applied without any concurrent afferent stimulation; in other words, during decerebrate tonus. The next four or five were applied while the crossed extension reflex was being evoked. The next three were applied during reflex inhibition, and the last three after cessation of reflex inhibition. It will be seen that, in all but the last series, the motor-nerve stimulus was supramaximal, and with these stimuli the contraction was regularly greater during inhibition than during decerebrate tonus, and greater during tonus than during the crossed extension reflex. Only with submaximal stimuli do the results become

TABLE 1

	EXCURSION	AVERAGE	RATIO
	mm.	*mm.*	*mm.*
During decerebrate tonus	13.3 13.0 13.0	13.1	
			1.38
During crossed extension reflex	12.9 12.5 11.0 9.5 9.5	9.5	
			1.35
During reflex inhibition	12.1 12.9 12.9 13.2	12.8	
			1.08
After cessation of inhibition	11.0 12.2 12.5	11.9	

irregular in this respect. The observations shown in this figure are samples of a large number of similar observations in this experiment.

The electromyograms in general supported the mechanical findings typified in figure 5. In some of the earlier experiments slight irregularities were found even with maximal stimuli, but in a large majority of cases the action currents conformed to the general rule; that is, those evoked during reflex inhibition were the largest, and those evoked during the maximum reflex activity were the smallest. In the last two experiments (November 17 and 26, 1924), in which the technique was most successfully developed, a large number of observations were recorded, and in every case in which the motor-nerve stimulus was maximal the size of the

action currents conformed consistently to this rule. In these experiments the results were treated quantitatively in the following manner; the excursion of the string of the galvanometer as registered on the film was measured in the case of every response to motor-nerve stimulation; in each group of responses the first three (during decerebrate tonus) were averaged; then the last two of those during the crossed extension reflex were averaged. This reflex, as has been shown by Liddell and Sherrington (1923), is characterized by a gradual increase to a maximum. Concurrent with this increase we usually found a progressive decrease in the size of action currents evoked by stimulating the motor nerve. The average of the last two excursions during the crossed extension reflex was taken as representing the maximum of reflex activity. Next the three excursions during reflex inhibition were averaged, and finally those after reflex inhibition had been withdrawn, representing a return to decerebrate rigidity, sometimes augmented by rebound. The ratio of change in the average size of excursions under these conditions was noted. A typical series of individual excursions thus treated is shown in table 1, in which the excursions are listed in the order of their occurrence. There were in these two experiments 48 complete series of responses in which the sequence described above was employed. The ratio of the response during the initial tonus to that during the maximum of reflex excitation varied from 1.05 to 1.52, averaging about 1.24. The ratio of change between the peak of reflex excitation and reflex inhibition varied from 1.01 to 1.44 and averaged about 1.22.[3]

In figure 6 is shown the action currents from a single series comprising those during decerebrate tonus, those during the crossed extension reflex, those during reflex inhibition, and one after the cessation of afferent stimulation. These action currents correspond with the twitches shown in the first group of figure 5. In figure 7 larger sections of the electromyogram of a similar group are shown, in order to give a better picture of the reflex background, as well as the responses to motor-nerve stimulation. It is interesting to note that the motor-nerve stimuli applied during decerebrate tonus, evoked, after the initial twitch, a clonic series of action currents showing a definite rhythm quite distinct from anything appearing in the uncomplicated tonus. This clonic effect appeared regularly throughout this experiment; it undoubtedly corresponds to the ankle jerk noted in figure 4.

[3] Throughout this experiment the last responses (after cessation of inhibition) were smaller than the first responses (before reflex excitation was begun), although there was little difference in the mechanical base line. This suggests a progressive decline independent of reflex activity, and may account for the ratio of change between reflex excitation and inhibition being apparently smaller than that between decerebrate rigidity and reflex excitation.

This experiment may be taken as typical of the behavior of the decerebrate preparation in this respect, but we must be guarded in generalizing too freely concerning the reflexes of decerebrate preparations. Striking individual variations occur, sometimes probably depending on uncontrolled experimental conditions such as blood pressure and position of head, sometimes probably representing individual peculiarities in the animal. Such individual differences have been noted in a number of previous researches (Cooper and Adrian, 1924; Adrian and Forbes, 1922; Forbes and Olmsted, 1925). One experiment should be especially noted. Some of the observations from it have already been reported by Forbes and Cattell (1924) in connection with another problem. The animal used in this experiment (January 24, 1921) showed little or no decerebrate tonus. Stimulation of the afferent inhibitory nerves caused no apparent relaxa-

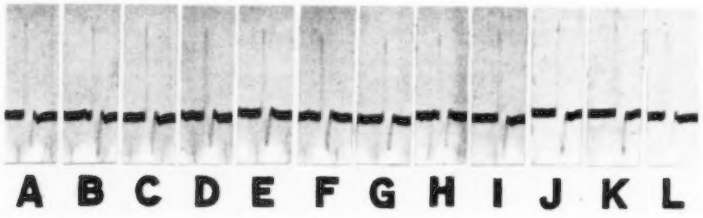

Fig. 6. Action currents of muscle in the first series of supramaximal motor-nerve stimuli shown in figure 5. November 26, 1924. All but the 12th and 14th responses in this series are shown. *A*, *B* and *C* during decerebrate rigidity; *D*, *E*, *F*, *G* and *H* during crossed extension reflex; *I*, *J* and *K* during inhibition; *L* during rebound. Hindle galvanometer; 2.75-μ, 17,000-ohm string; magnification, 490; string tension 86.8 m. per amp.

tion of the muscle. Therefore we devoted our attention to comparing the condition during this atonic state with that during the crossed extension reflex. Mechanically this reflex was vigorous; electrically the responses of the extensor muscle, as recorded by the galvanometer, were unusually small. This contrast has already been shown in figures 10B and 13 of the paper by Forbes and Cattell (1924). Another series of mechanical responses to motor nerve stimulation before and during the crossed extension reflex, similar to that appearing in the previous paper, is shown in figure 8. The corresponding electric responses are shown in figure 9. The large mechanical effect during the sustained reflex, involving greater shortening of the muscle than in the maximal twitch, contrasts strikingly with the small size of the corresponding electrical excursions, which amount to only about one-thirtieth of the excursion marking the neuromyal twitch. In this experiment a comparison of the size of the action currents

evoked by motor-nerve stimuli during activity and inactivity of the reflex center, shows very little change. The ratios of the average excursions in the two conditions varied from 1/1 to 1/1.12. This preparation presents a striking contrast with that which furnished the electromyogram in figure 4. In this case (fig. 9) the size of the excursions accompanying the vigorous crossed extension reflex are much smaller than those in the other (fig. 4) accompanying the less vigorous rebound contraction after inhibition; also reflex excitation caused much less diminution in the size of response to motor-nerve stimulation in this case than that appearing in

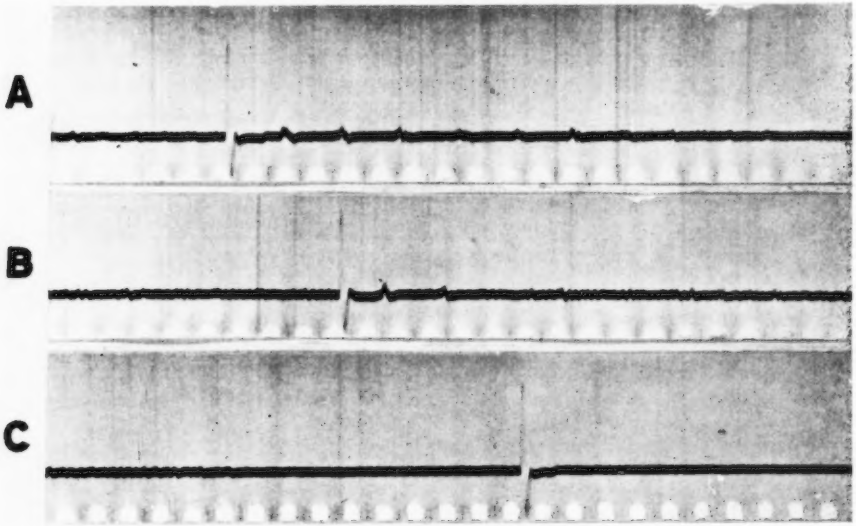

Fig. 7. Electromyograms from the second series of responses shown in figure 5, showing reflex activity compared with responses to motor-nerve stimuli. November 26, 1924. *A* corresponds with the second contraction of the group; *B* with the seventh; *C* with the ninth. Electrical recording conditions as in figure 6.

figure 4. Both facts signify that in this preparation (fig. 9) fewer fibers were in action at any given moment; yet their mechanical effect was large.

The question arises whether the mechanical effects of contraction may so modify the action currents as to confuse their evaluation as evidence of the proportion of fibers that are accessible to the stimulus. Forbes, Ray and Hopkins (1923) recorded action currents in the frog's gastrocnemius and sartorius muscles in the case of the simple twitch, under various degrees of initial tension. In the case of the gastrocnemius the action current increased in size when initial tension was increased, but with the

sartorius no such increase was observed. It was suggested that the increase in the case of the gastrocnemius muscle might be due to the obliquity of the fibers.

Fulton (1925) has also investigated this point and extended his observations to include action currents during tetanus as well as in the simple twitch. He confirms the observation of Forbes, Ray and Hopkins on the gastrocnemius. He also finds that if a muscle is allowed to shorten in a brief tetanus, the action currents decrease in size during the shortening, but this decrease ceases when the plateau of contraction is reached. If in a parallel-fibered muscle the contraction is rendered perfectly isometric

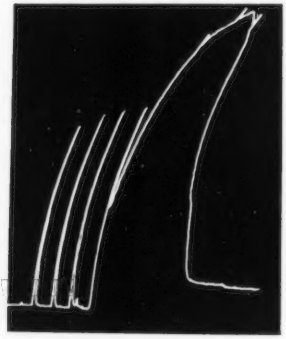

Fig. 8

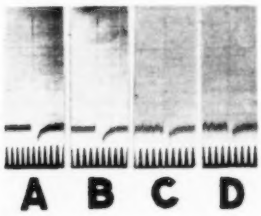

Fig. 9

Fig. 8. Record of atonic preparation showing reflex contraction greater than single maximal twitch. January 24, 1921.

Fig. 9. Electric responses of 1st, 2nd, 5th and 7th contractions (in response to motor-nerve stimulus) shown in figure 8. *A* and *B*, before beginning of reflex, *C* and *D*, during reflex. January 24, 1921. Motor-nerve stimulus, 113 Z units in all.

Hindle galvanometer; 1.5-μ, 19,500-ohm string; magnification, 490; string tension, 39.8 m. per amp.

the decrease in the size of action current disappears. He therefore associates the decrease in the action current with shortening of the fibers.

Fulton's results would lead us to expect during reflex excitation a decrease in the size of the action currents observed in our experiments. One of his observations on a mammalian muscle shows a decrease of the action current to about 58 per cent of its initial value in a muscle contracting almost isometrically, but starting from small initial tension (Fulton, 1926, fig. 79). In our experiments the contractions were isotonic, the load in all cases being comparatively light. We may conclude that such a large decrease in the action current of the neuromyal twitch during

reflex activity as that shown in figure 4 (1/0.31), could not be explained entirely by mechanical shortening, but must signify that many of the motor units were refractory. A small change, such as that in figure 6, might be due to the mechanical effects shown by Fulton, and is, therefore, ambiguous in its bearing on our problem. Evidence on this point is found in the experiment of January 24, 1921 (figs. 8 and 9) which showed very little change in the size of the action currents evoked by motor-nerve stimulation, even though the reflex contraction was large and the mechanical effects must have been almost as great as in any of our experiments. From this we may argue that in such experiments as that illustrated in figure 6, or in figure 4, in which the mechanical effect was smaller, the marked reduction of the action currents evoked during reflex activity could hardly be explained by the mechanical effect alone, and therefore signifies refractory fibers.

In view of uncertainty as to the amount of overshoot in the isotonic lever and the effect of shortening of the muscle fibers on the size of the action current, we performed six experiments with the isometric lever. Fulton's previous experiments have shown that the more perfectly isometric the contraction, the smaller was the decline in the size of successive action currents during tetanization. Therefore, by recording the reflex contraction under approximately isometric conditions we could hope to minimize the confusion due to shortening. In each record we applied two approximately equal supramaximal stimuli to the motor nerve, one before and one during the crossed extension reflex. In order to control the equality of the stimuli we repeated the observations without the reflex. The experiments were performed under varying degrees of initial tension. To control the effect of tension development on the size of the action current we also tetanized the motor nerve in most experiments. The resulting tetanus of the muscle developed far greater tension than was obtained in any reflex response.

In the mechanical records the increment of tension resulting from the motor nerve stimulus is markedly less when applied during reflex activity than when applied before the reflex. Examples of this are shown in figures 10 and 11. The controls to show the equality of response to the two motor-nerve stimuli in absence of reflex stimulus are not always perfect because the neuromyal twitch was followed by a small stretch reflex ("myotatic appendage," Fulton, 1926, p. 278, p. 527) which often persisted up to the time of application of the second stimulus. However, when this residual tension was small the actual rise of the lever was almost the same for each stimulus, showing that they were both supramaximal (see fig. 10B). Mechanically, therefore, the isometric records corroborated those made with the isotonic lever.

Turning to the action current records, let us consider first the controls

made by tetanizing the motor nerve with the muscle attached to the isometric lever. In some experiments there was no appreciable decline in the size of action currents as the tension increased. In some experiments

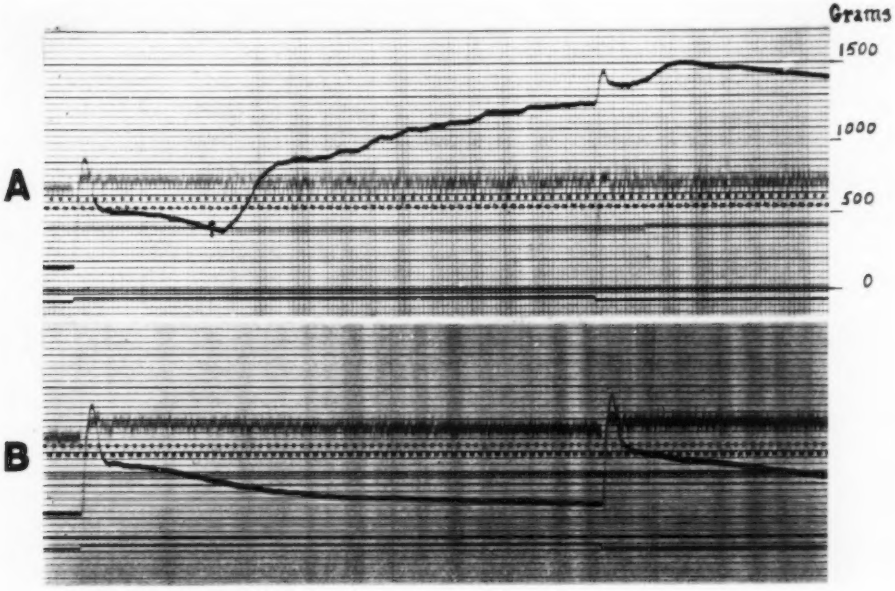

Fig. 10. *A*, Isometric records of contraction in response to single faradic stimuli applied to uncut motor-nerve before and during crossed extension reflex; *B*, control experiment showing equality of responses to the two stimuli in absence of reflex. Time shown in *A* by fine vertical lines at intervals of 0.02 second. Both plates at same speed. From above downwards (excluding the horizontal mm. lines) are the shadows of: 1, the galvanometer string (magnification × 300; tension 32 m. per amp.; shunt, 5000 ohms; resistance, 3,800 ohms); 2, vibrator showing rate of crossed reflex stimulation (28 per second; break shocks only; Harvard coil, coreless, with secondary at 6 cm. and 5 ohms in primary which was fed by 2 v. accumulator); 3, short-circuiting key showing duration of reflex stimulation (opening of key obscured by myograph, see arrow). 4, Myograph (23 mm. = 1000 gm.). 5, Line of zero tension of myograph. 6, Key which delivers faradic stimuli to uncut nerve (coreless Harvard coil fed by 0.167 amp. with secondary at 2 cm.; threshold for break shock was 10 cm. so both stimuli are well over-maximal). The slow speed of the plate prevented clear definition of the string excursion. (February 25, 1927.)

with comparatively slight initial tension a moderate decline occurred. In one case the action current declined to 55 per cent of the initial size. In all these cases the tension developed during the tetanus was much greater than in any reflex.

When the responses to motor nerve stimulation before and during the reflex were compared the usual decrease in the size of action current was regularly found. In some cases it was very slight, but in most cases it amounted to between 10 and 25 per cent. An example of this observation is shown in figure 11. Comparing this decrease with the decline during motor nerve tetanus, which was usually smaller, even when much greater tension was developed, this result reinforces our main conclusion that the decrease in size of action current during the reflex could not all be explained as due to the mechanical shortening, and therefore signifies the preoccupation of the motor units.

Discussion. The evidence in our experiments seems to show clearly the effect of preoccupation in the neuro-muscular units during reflex activity, rendering them refractory to stimuli applied directly to the motor nerve. This supports the view that the impulses of reflex origin are

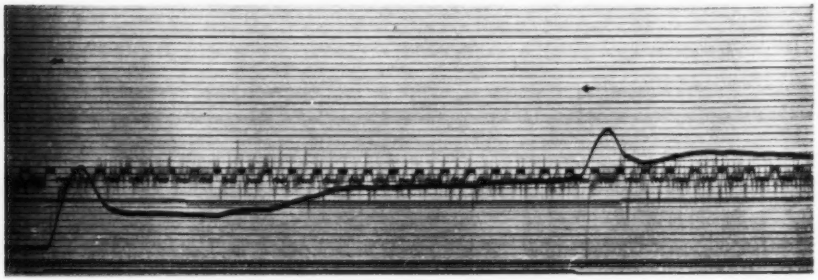

Fig. 11. Same as figure 10 but taken on a faster plate to show string excursion (see arrows for peak). The shadow of the vibrator (20 per sec.) is superimposed on that of the string. Galvanometer not shunted.

essentially the same as those set up by artificial stimulation; not only do they cause similar contractions and action currents, but they render the tissues refractory to further stimulation in the same way.

As to the question whether there is any essential difference in the kind of activity or the character of neural discharge involved in the various reflex contractions under consideration: decerebrate tonus, post-inhibitory rebound and crossed extension reflex,—all we can say is that our methods of attack revealed no differences in kind, only differences in degree. If there were a great difference in the frequency of motor-nerve impulses between the different types of activity we should expect equal motor-nerve stimuli to evoke different degrees of contraction, although starting from the same base level, according to the character of the reflex background. Slight differences of this sort may exist, but they are not large enough nor consistent enough to warrant any conclusion that we are

dealing with fundamentally different types of reflex activity. Our results give the impression that decerebrate tonus, rebound and crossed extension reflex are essentially alike as regards the character of the neural discharge from the center. Certainly all three are alike in their capacity to render the neuro-muscular units refractory to motor-nerve stimulation.

It is interesting to note that Hoffmann (1918) in connection with another problem, has shown electromyograms revealing the same effect which our experiments have shown. Action currents in the muscle, evoked by motor-nerve stimulation during reflex inhibition, were notably larger than those evoked during reflex excitation.

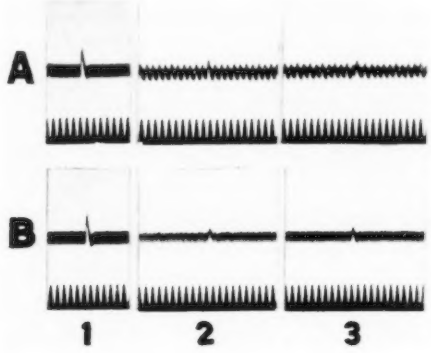

Fig. 12. Control experiment with high-frequency stimulation of motor nerve substituted for reflex. Action currents of external interosseous muscle. *A*, stimulus frequency, 110 per second; *B*, 410 per second. In each case, *1*, response to single stimulus in resting nerve-muscle preparation; *2* and *3*, responses during high frequency stimulation at proximal electrodes. February 7, 1924. Hindle galvanometer; 2.75-μ, 17,000-ohm string, string tension 104.1 m. per amp.

Note that with stimulus-frequency 410 per second, muscle responses appear at half that frequency, although it was shown with the galvanometer that the nerve fibers were responding to every stimulus.

In order to demonstrate the effect to be expected from keeping a motor nerve occupied with impulses of various frequencies we carried out two control experiments. The popliteal nerve was cut at the hip and stimulating electrodes were applied in the thigh near the cut end. All branches except the tibial, which innervates the external interosseous muscle, were cut; a second pair of stimulating electrodes was applied to the tibial branch in the lower leg. Leads were applied to the external interosseous muscle and connected with the string galvanometer. A writing lever was arranged to record its contraction on a smoked drum. The proximal pair of stimulating electrodes in the thigh was connected with an

inductorium whose primary circuit included the rotary interrupter. This was for the artificial duplication of hypothetical reflex discharges at various frequencies. The distal pair of stimulating electrodes in the lower leg was connected with an inductorium arranged for single break shocks, representing the motor-nerve stimulation in our reflex experiment. Both mechanical and electrical records show certain facts of interest. In some tests the rotary interrupter was run at a speed giving 110 stimuli per second, in others it delivered from 410 to 460 stimuli per second. Both submaximal and supramaximal stimuli were applied at the distal stimulating electrodes. The mechanical records showed that at either frequency of the rotary interrupter complete tetanus occurred and the single stimuli applied farther down the nerve, whether supramaximal or submaximal, produced no visible mechanical effect whatever. In the electrical records submaximal stimuli rarely or never evoked responses which could be distinguished from those already occurring in response to tetanization. Supramaximal stimuli, on the other hand, evoked well-defined galvanometric excursions which stood out clearly from the background of preëxisting activity in the muscle. Examples of this at two frequencies are shown in figure 12. The probable explanation for their appearance, even though the motor nerve was already being tetanized and causing a maximal contraction in the muscle, is probably due to the difference in velocity of conduction of the different fibers in the motor nerve. In the course of conduction these impulses lose their synchronism and the muscle action currents are somewhat out of phase with each other in the different fibers. A supramaximal stimulus applied nearer to the muscle sets up at once a synchronous volley of impulses in all fibers except those in the absolute refractory phase at the moment. The resulting muscular responses will be more nearly synchronous than those evoked by the tetanizing stimulus applied higher up, and a well-defined peak in the electromyogram will result.

One fact stands out clearly from the mechanical records—it is impossible by this method to duplicate the condition obtained during a sustained reflex contraction. Complete maximal tetanus prevents further contraction in the muscle. The fact that twitches could always be evoked by motor nerve stimulation, even during the height of a crossed extension reflex, shows that at no time was there complete tetanus of all the muscle fibers innervated by the motor nerve (cf. Camis, 1909; Cooper, Denny-Brown and Sherrington, 1926). Indeed the smallness of the change in the action current evoked by motor nerve stimulation shows that often, even during a vigorous reflex, a very small percentage of the muscle fibers are in action at a given moment.

SUMMARY

1. If reflex activity were mediated by impulses different in kind from those evoked by the electrical stimulus, or if decerebrate tonus involved a different mechanism or a different kind of function from other reflex contraction, we should not expect these activities to render the motor nerve fibers refractory to artificial stimuli.

2. In the present experiments the mechanical and electrical responses of an extensor muscle in the decerebrate cat, evoked by single stimuli applied to its motor nerve are compared with one another during various forms of reflex activity of the extensor muscle; namely, decerebrate tonus, post-inhibitory rebound, the crossed extension reflex and in reflex inhibition of the extensor center. We find that the response to the motor-nerve stimulus is larger during inhibition than during reflex excitation and that the greater the reflex excitation, the less the response to the motor-nerve stimulus. Apparently during reflex activity a certain percentage of the motor nerve fibers or the muscle fibers they innervate, are rendered refractory to stimuli applied directly to the motor nerve. If the refractory state is in the nerve fibers it shows an essential resemblance between the reflex impulses and those artificially produced.

3. Even a vigorous reflex usually causes but a small decrease in the response to a motor-nerve stimulus; this corroborates the observations of Sherrington and others as to the small proportion of fibers in a muscle which take part in its reflex contraction.

4. This method revealed no apparent qualitative difference between tonus and the extensor reflexes.

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DEMONSTRATION OF THE NATURE OF THE ARTERIO-VENOUS MESHWORK IN THE FROG'S KIDNEY

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The apparent double nature of the kidney has given rise to considerable discussion and experimentation to determine what part in urine formation each unit performs.

Owing to the recent excellent account of the literature on the subject by Marshall, it should be unnecessary to go into detail discussing this literature.

The Bowman-Heidenhain theory postulates that the capsule has essentially the same function as the tubule. The Neo-Ludwig theory assumes that the capsule secretes all the non-colloidal contents of normal urine by filtration in dilute form, and that the glandular cells of the tubule reabsorb all but the normal urinary constituents. The "Tubule cum Rete" theory held by Lamy, Mayer and Woodland assumes that all the constituents of urine, in the concentration found in the bladder, are secreted by the tubules, and that the function of the Bowman's capsule is to decrease the blood pressure without decreasing the blood volume. The "modern theory" advocated by Cushny is really a modification of Ludwig's scheme of filtration and reabsorption, supplementing it as far as necessary by the "vital activity" of the tubule cells as postulated by Heidenhain. Cushny advances the idea that reabsorption by the tubules is not the passive diffusion which Ludwig believed it to be, but active absorption entailing expenditure of energy by the cells. To explain the different concentrations of the various urinary constituents as compared with the blood, he introduces the idea of "threshold" substances and "no threshold" substances. Threshold substances are actively absorbed by the tubule cells and returned to the blood while no threshold substances, such as urea, are rejected.

Many physiologists have regarded the Neo-Ludwig theory as offering the best explanation of the function of each unit of this excretory apparatus, though there has for some time existed evidence that the glandular cells of the tubules, as shown by dye experiments, actually secrete from the blood into the lumen of the tubule and not from the lumen into the blood as the theory supposes.

The "Tubule cum Rete" theory assumes that the act of urinary secre-

tion is entirely a tubular function, and that the function of the glomerulus is to reduce the blood pressure and rate of blood flow without reducing the amount of blood in the capillaries around the tubule. It further assumes that the inclosure of the glomerulus within Bowman's capsule prevents filtration of the urinary constituents and controls the rate of flow of blood through the kidney. This last theory is an exact antithesis of the Neo-Ludwig theory, and is in reality a greatly modified view of the Bowman-Heidenhain theory.

On account of the fact that when the renal artery is obstructed so as to reduce the blood pressure in the kidneys to 30 mm. or less, and on account of the general parallelism between blood pressure and kidney secretion (supported by various experiments by Goll, Herman, Bibbot and a host of others) Bayliss said in 1915 that the evidence for glomerular filtration is overwhelming, and this is the viewpoint given in all of the textbooks which I have examined.

In spite of this there is much in recent experiments on the kidney that could be explained better on the basis of a modification of Heidenhain's conception.

It would seem that in order to determine the validity of any of these viewpoints it would be necessary to determine the nature of the renal venous and arterial meshwork. This was undertaken and frogs were used, for in many of the lower vertebrates the renal portal vein breaks up at the kidney, and entering it, emerges at the post caval vein, its blood here being mixed with the blood from the renal artery. The artery entering the kidney from the median side undoubtedly breaks up into capillaries of the tubule.

If this were true, then it would be possible to use any animal, such as a frog, having this double circulation to the kidney, to study separately the function of the capsule or of the tubule by obstructing either the renal artery or the renal portal vein and observing the rate of flow of urine, and comparing the chemical analysis of the urine in each case. Nussbaum undertook this, but on account of the small European frogs which he used, he was able to obtain only very small quantities of urine.

Nussbaum, and later Bainbridge and Beddard, noted that tying off the renal artery stopped the formation of urine. Then, injecting urea, they found that about one-fourth the amount of urine was secreted as that obtained from normal frogs following the same dose of urea.

Method of procedure. The original intention was to repeat this experiment of Nussbaum's, using large bull frogs, but as a preliminary test the following experiment was devised: first, to find the amount of anastomosis between these two systems of blood vessels, and second, to discover the extent to which these systems break up into capillary beds. For if there is just one secreting set of units in the kidney of the frog, the double

circulation would become united in function, and Nussbaum's idea (fig. 1) of the possibility of solving the individual function of the glomeruli and of the tubules by ligating the renal artery or the renal portal vein, obtaining samples of urine produced in each case, would be without meaning, and if, as Woodland claims, the renal portal veins do not break up into capillaries, but pass through the kidney in channels (fig. 2) which, so far as the positive side of urine secretion is concerned, are functionless, then Nussbaum's conception is erroneous.

The following perfusion experiments were carried out in the hope of securing additional information on this point.

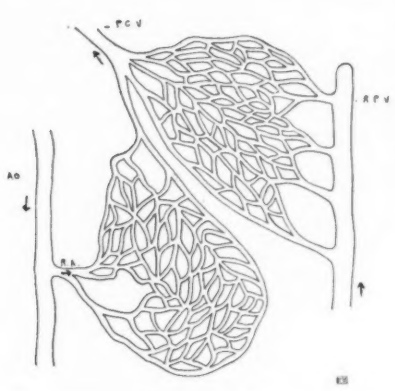

Fig. 1

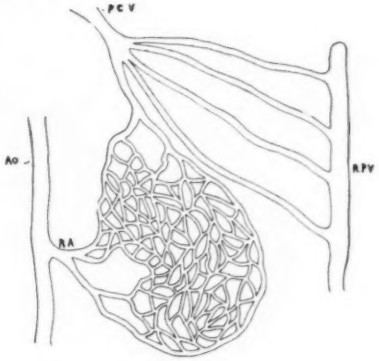

Fig. 2

Fig. 1. Diagrammatic drawing showing the concept of Nussbaum, i.e., practically an independent capillary bed for the renal artery, and another for the renal portal vein.

Fig. 2. Diagrammatic drawing showing the concept of Woodland, i.e., a capillary bed for the renal artery, but no capillary bed for the channels from the renal portal vein.

A cannula was introduced (fig. 3) into each of the renal portal veins, one into the aorta just anterior to the kidney, and one into the post caval vein to lead off the perfusion fluids. The fluid used was aerated frog Ringer's solution, and the frogs were Rana catesbiana which had been pithed and the spinal cords destroyed to prevent reflex action. The animals were kept under as nearly the same condition of temperature and moisture as possible, after the perfusion fluid had been allowed to flow through, until the blood had been entirely removed and the outflow had become constant.

Test with adrenalin. Adrenalin in suitable concentration (1 part to

20,000 or 1 part to 100,000) was introduced, the amount and concentration being the same for each experiment.

If, as Nussbaum thought, the inter-renal meshwork is composed of two sets of capillaries (fig. 1), then injection of adrenalin into either the aorta or the renal portal veins would produce an approximately equal slowing up of the drop rate from the post caval vein but if Woodland's

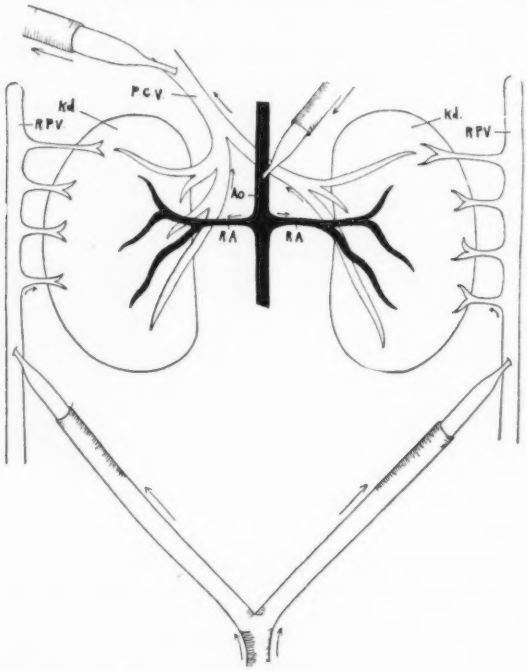

Fig. 3. Diagrammatic drawing showing the location of cannulae in the blood vessels for injecting the kidney of the frog.

Ao., aorta; *Kd.*, kidney; *P. C. V.*, post caval vein; *R. A.*, renal artery; *R. P. V.*, renal portal vein.

conclusion be true, that the renal portal veins do not break up into capillaries, then an injection into this system would not produce as great a decrease in the drop rate as the same amount of adrenalin injected into the tube leading to the aorta because, for a given amount of fluid passing through a capillary bed a great deal more surface would be exposed to absorb the adrenalin than would be exposed in a single large blood vessel, or even in a few moderate sized ones. According to Krogh, there has been no direct observation of the effect of adrenalin on the capillaries of the

frog's kidney, but no evidence given that would contradict the above conclusion.

In each case, after the flow had become constant, the injection was made without disturbing the arrangement of the tubes, and the drops recorded until the minimum was reached and recovery had begun. After complete recovery to normal flow, an injection was made into the other system. These injections were made into rubber tubes above the cannulae, 6 cc. of one part adrenalin to 100,000 parts frog Ringer's solution, in the first series of frogs. This, of course, was further diluted by the solution in the tube, but dilution should have been approximately the same in all the tests. Thirty-eight frogs were used in these tests in two series. In the first a simultaneous flow was maintained in both the arterial and venous systems while injection was made in first one, then the other, as long as results could be obtained. In the second series only the blood vessels to be injected were cannulated. The following tables give the results obtained:

Table of the first adrenalin series (14 frogs)

Average rate of normal flow before aortal injection	66.1 drops	
Average rate of flow after injection into aorta	42.3 drops	
Average decrease	23.8 drops	or 36 per cent
Average rate of normal flow before R.P.V. injection	68 drops	
Average rate of flow after R.P.V. injection	58 drops	
Average decrease	10 drops	or 14.8 per cent

The second series, the aortal injection only was made on one set of frogs and the decrease noted; in another set, the renal portal vein only was used for injection and results were again noted. One part of adrenalin to 20,000 parts frog Ringer's solution was used in this series. The results were as follows:

Table of averages from the second series of tests (24 frogs used)

Average rate of normal flow before aortal injection	39 drops	
Average rate of flow after aortal injection	18 drops	
Average decrease	21 drops	or 53.8 per cent
Average rate of normal flow before R.P.V. injection	106 drops	
Average rate of flow after R.P.V. injection	76 drops	
Average decrease	30 drops	or 28.3 per cent

In addition to the typical tests recorded here, this same experiment has been demonstrated a large number of times to classes and to visitors to the laboratory, but at no time after the injection of the adrenalin into the renal portal veins has the decrease in the drop count been as great as that following an injection of an equal amount of adrenalin into the aorta. At one time, before injecting into the renal portal vein, a bubble of air was accidentally let into the cannula and was carried by the current into the vein. This bubble was observed clearly as it passed through the kidney without breaking up. It was visible from the time it appeared in the cannula going into the renal portal vein until it disappeared into the rubber tube attached to the cannula from the post caval vein, and no

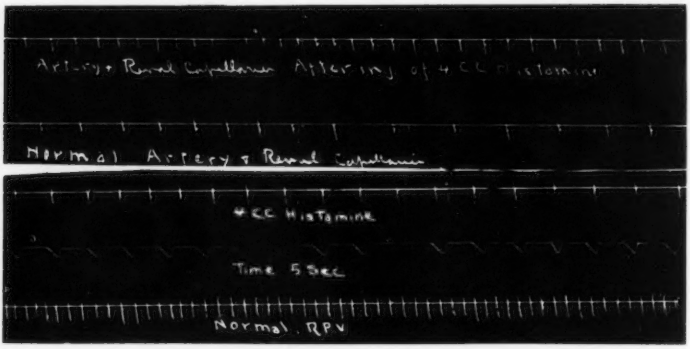

Fig. 4. Kymograph record showing the change in drop rate produced by injecting histamine from the renal artery and its capillary bed as compared with the change of rate from the renal portal vein. The normal flow of blood from the renal artery, as shown here, was fourteen drops per minute, while the flow after injecting 4 cc. of 0.05 per cent histamine was 28 drops per minute. The normal flow from the renal portal vein was 39 drops per minute, and the flow after injecting the same quantity of histamine was 13 drops per minute.

change took place in its shape or size other than such as was necessary to make it conform to a rather large channel. *Certainly it did not pass through a capillary bed.*

Test with histamine. In order to check up on the adrenalin tests and to determine the effect of histamine itself, histamine was used in similar perfusion experiments. The solution used contained $\frac{1}{2}$ mgm. of histamine per cubic centimeter in frog Ringer's solution. In all, 23 frogs were used in this series with varying quantities of histamine. In all cases an injection of histamine 1 mgm. or more into the arterial system caused an increase in the drop rate, while a similar injection into the renal portal veins caused a decrease in the drop rate. Kymograph records were made of

this (fig. 4) and the following tables show the results obtained from the 23 frogs used in this test.

Tables of averages from the histamine series (23 frogs)

Average rate of normal flow before aortal injection	12.4 drops	
Average rate of flow after aortal injection	31.3 drops	
Increase of drops per minute	18.9 drops	or 151.4 per cent
Average rate of normal flow before R.P.V. injection	48.1 drops	
Average rate of flow after R.P.V. injection	31.8 drops	
Decrease in drops per minute	16.3 drops	or 33.9 per cent

In trying to analyze the results obtained above, resort was had to Inchley's work with guinea pigs in which he found that histamine in the concentration used here, showed no effect when injected into an artery, but when injected into a vein a slowing up of drop rate was recorded. This was found to be true of the frog also. Two points along the abdominal vein and two points in the aorta were cannulated and perfused with Ringer's solution, normal drop count taken, and then varying doses of histamine were used, and no change was shown in the drop rate from the artery, while the drop count from the vein decreased in proportion to the amount of histamine used (fig. 6, graph 1).

When injection was made into the artery and capillary bed of the kidney and the injection into the renal portal vein of the kidney the venous injection in graph 2 shows the same results as in graph 1, and indicates that it has no capillary bed while the arterial flow shows a decided increase which must be due to the influence it has upon the capillaries in the kidney.

Injection of air, mercury and paramecia. Air injected into the renal portal system, as previously noted, was observed to pass through the kidney in comparatively large channels. Mercury passed through and came out into the cannula on the post caval side and a suspension of Paramecia could be observed very readily as they passed through the kidney, all proving the existence of channels from the renal portal veins through to the post caval system, *but none of these substances could be gotten to pass through the renal artery and out into the post caval vein.*

Injections with India ink. If India ink is injected into either the renal artery or the renal portal vein it will inject all parts of the kidney, but when injection was made into the renal portal veins while maintaining a strong flow of Ringer's solution through the renal arteries, only the principal channels of the veins were injected, and these, contrary to Woodland's concept, showed much branching to minor parts of the kidney. This observation led to the conclusion that there was a great deal of anastomosis between the two circulatory systems.

Direct observation of the kidneys. A modification of Richard's method of illuminating the kidney was used, the frog being placed on the microscope stand which was tilted to get suitable lighting. Thread loops were placed under the renal arteries and renal portal veins so that they could be pulled at any time to decrease or cut off the circulation from either system.

While the channels from the renal portal veins could be traced entirely through the kidneys, very different views were obtained from those pictured by Woodland, for the view changed many times and other vessels

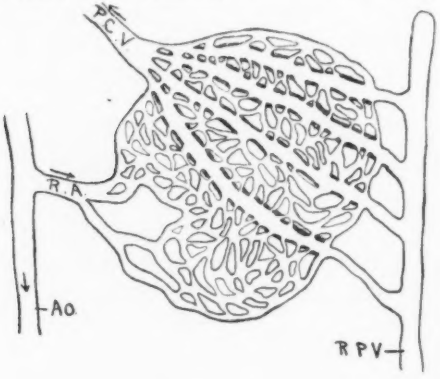

Fig. 5

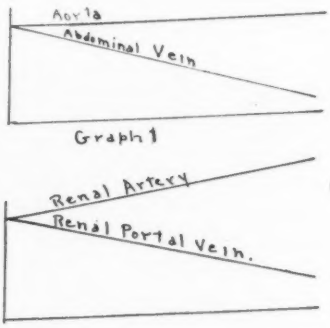

Fig. 6

Fig. 5. Diagrammatic drawing showing the nature of the circulation in the frog's kidney. The channels from the renal portal vein go all the way through to the post caval vein, but anastomosis with the capillary bed occurs at very frequent intervals.

Fig. 6. Graph 1. The drop rate from the aorta of the frog shows no increase nor decrease after the injection of any amount of histamine, while the drop rate from the abdominal veins shows a decrease that is proportionate to the amount of histamine used.

Graph 2. The drop rate from the post caval vein, when the injection was made into the renal artery (fig. 3) shows increase proportionate to the amount of histamine used, while the drop rate from the post caval vein, when the injection was made into the renal portal vein, showed a decrease proportionate to the histamine used.

from the arteries entered at such frequent intervals one could hardly imagine a more complete anastomosis. The flow normally seemed to come from the arteries filling all the capillaries and flowing out into the channels, but the least amount of pressure on the renal artery caused the flow to reverse itself and to flow from the renal portal vein out through all the capillaries into the capsules and around the tubules. This led to the conclusion that one might tie off the renal artery on the renal portal vein and the kidney would function as before.

Obstruction of renal arteries and of renal portal veins in frogs and toads.

The frogs with the renal arteries tied off lived as long as the other animals similarly operated upon, usually, from 14 to 16 days, but one lived 20 days and was killed to see if the renal arteries were completely obstructed. India Ink injected into the aorta did not inject the kidney. Urinary analysis showing no difference from the normal. Toads, which were found to have kidneys with the same double circulation as the frogs, were much better for this purpose as they could live under laboratory conditions for a considerable length of time. Nine toads were used, in 5 of them the renal arteries, and in 4 the renal portal veins were ligated; one of the latter died within two days, but the other eight lived until killed for post-mortem, a period averaging 20 days. The amount of the urine and its nitrogen content did not vary consistently from the normal toads that had not been operated on. These toads were operated upon on August 5, 6 and 7, and were killed and a post-mortem made on August 26. In all cases but one the obstruction in the blood vessels had been complete, as determined by injection of India ink. In this one case one branch of the renal artery had not been tied off and had continued to supply a part of the kidney; that part of the kidney, however, had no different appearance than the rest of the organ, and this toad seemed to be in no better condition than the other seven toads in which obstruction had been complete.

Discussion. It seems that in the toad and frog, due to the complete anastomosis between the two circulatory systems, that either the arterial system or the venous system is adequate to supply, in the absence of the other, all the blood necessary to the kidney to enable it to carry on its work normally. Then we have a very different concept of the nature of the circulation in the frog's kidney from that of Nussbaum (fig. 1) where you have two practically independent capillary beds and from that of Woodland (fig. 2) where the channels from the renal portal vein flow through, practically independent of the capillary bed, but rather as shown in figure 5, which gives diagrammatically a true picture of the relation between the two circulations in the kidney from which it can readily be seen why either would function adequately in the absence of the other.

The author wishes to thank Dr. A. J. Carlson for assistance, criticism and the use of his laboratory, and Dr. Carl Hartman for suggestions and aid in the preliminary experiments.

CONCLUSIONS

1. The blood vessels from the renal portal veins in the frog do not break up into separate capillary beds and supply the tubules or any other portion of the kidney with blood, but pass through the kidneys in relatively large channels which anastomose with the renal arterial system.

2. The perfusion experiments, founded on Nussbaum's conception of the circulation through the frog's kidney, by Miss Cullis, by Bainbridge, Menzies, Collins, Rowntree, Geraghty and Richard and his co-workers, in so far as the renal portal vein is concerned, did not perfuse the kidney in the sense of supplying the tubule through a capillary bed, and the conclusions of Cushny (*The secretion of urine*, pp. 79–83) drawn from some of these results, are without basis.

3. Very complete anastomosis occurs between the two systems of blood vessels in the kidneys of the frog so that any interference with the circulation of either the arterial or venous system will cause the unobstructed blood stream to supply the entire renal capillary system.

4. Frogs and toads continue to live and excrete normal urine with either the renal arteries or the renal portal veins tied off.

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THE EFFECT OF ANOXEMIA ON THE SIZE OF THE HEART AS STUDIED BY THE X-RAY

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Kaufmann and Meyer (1) (1917) clinically studied the hearts of soldiers who had returned from campaigns in mountainous districts, and reported that the hearts of these soldiers were greatly increased in size; but these enlarged hearts decreased in size with short rests only, or more quickly after treatment with digitalis.

Whitney (2) working at Mineola found by percussion a considerable dilatation of the hearts of aviators while they were subjected to anoxemia. He states that of 10 medical officers subjected to anoxemia by rebreathing, 5 of them developed marked cardiac dilatation; one corresponding to an altitude of 14,000 feet (about 12 per cent oxygen), one to 16,000 feet, two to 18,000 feet and one at 20,000 feet.

LeWald and Turrel (3), working on aviators and using the x-ray, reported only a slight increase in the size of the cardiac silhouette in some cases, and on the other hand, some showed a slight decrease; in no case was there a pronounced dilatation.

Barcroft et al. (4) ascertained no cardiac enlargement as measured by the x-ray at an altitude of 14,000 feet. In three cases it was found that the heart was smaller than at sea level.

Somerwell (5) reported that all the men who went higher than 27,000 feet had dilated hearts, from which it took from one to three weeks to recover.

Takeuchi (6) in 1925 found that anoxemia caused an immediate enlargement both in length and width of the heart in cats. He made his observations by opening the chest, administering artificial respiration by an appropriate apparatus in which the amount of oxygen could be regulated. The changes in the size of the heart were then studied by means of the cinematograph.

Jarisch and Wastl (7), using the cardiometer, found a distinct cardiac dilatation in anoxemia.

Gremels and Starling (8), using the heart-lung preparation, reported

[1] Laura Doone Donnelly Fellow in Physiology.

that the cardiac dilatation is most pronounced below 10 per cent oxygen and at the same time other signs of cardiac failure appeared.

The present work was undertaken because of the conflicting observations on man, and the unphysiological experimental conditions in most of the studies on animals. The dog and the cat, because of the position of the diaphragm, give better x-ray silhouettes of the heart than does man, and it is possible in animals to employ greater degrees of anoxemia.

PROCEDURE. The anoxemia was induced by reducing the atmospheric pressure in a steel respiratory chamber described by Kolls and Loevenhart (9). A large mercury manometer mounted on a large meter stick was connected to the chamber by pressure tubing, so that the pressure in the chamber was read directly in millimeters of mercury and converted to oxygen percentage. A Crowell rotary pump, run by $\frac{1}{2}$ H.P. motor was used to reduce the pressure in the chamber. The speed of withdrawal of the air was regulated at will by valves in the outflow and inflow tubes. The inlet valves of the chamber were so arranged that it took from 6 to 8 minutes to reach the desired pressure and the animal was kept at this pressure from 3 to 4 minutes; these conditions were kept approximately uniform in all the experiments.

Dogs were trained to lie quietly on their backs on an appropriate animal board during the 1 to 5 second x-ray exposure. The distance from the center of the tube to the film was kept at one meter. The animals were then placed in the respiratory chamber. The removal of the animal from the chamber and taking the x-ray picture required from 20 to 30 seconds. It was aimed to place the animal in a position identical with the control. As it was practically impossible to train the rabbits and guinea pigs to lie on their backs, appropriate animal boards were made to which these animals could be fastened and they were in this way both x-rayed for control and placed into the chamber.

In the remainder of the work barbitalized dogs and cats were used. Barbital was given in doses of 250 to 280 mgm. per kilo body weight. As a rule it was given intravenously, although in a few cases it was given by means of a stomach tube.

In the beginning of the work several pictures were taken of the animal in the course of a day and on two animals pictures were taken on different days. It was soon found, however, that the percentage of difference in the area of the cardiac silhouette was so small that it was not deemed worth while to take more than one or two control pictures. Some of the unanesthetized animals were placed in the chamber several times so that several pictures were made on the same animals; in the table of results, however, only the average figure is given.

It was realized that criticism could be made of the results obtained, as it took 20 or more seconds to remove the animals from the respiratory

chamber and place them under the x-ray tube. It is also admitted that it is nearly impossible to place them in exactly the same position for the two photographs. In order to check the results in the above method anoxemia was induced by an appropriate mixture of oxygen and nitrogen in an apparatus ordinarily used for clinical administration of nitrous oxide oxygen. The tracheal cannula of the animal was connected directly with the apparatus, so it virtually was a closed system, except for the fact that a type of flutter valve was inserted near the tracheal cannula, so that the expired air could not re-enter the otherwise closed system. Approximately the same percentage of oxygen was used as in the respiratory chamber and the results obtained were, within a small per cent of experimental error, the same.

The area of the heart shadow was computed by sketching the radiographic silhouette by means of a pen and then measuring the area by a planimeter, as described by Bardeen (10).

RESULTS. The following table gives the results obtained:

ANIMAL	WEIGHT	CARDIAC AREA		INCREASE IN CARDIAC AREA IN ANOXEMIA	OXYGEN	COMMENTS
		Normal	Anoxemia			
	kgm.	*sq. cm.*	*sq. cm.*	*per cent*	*per cent*	
Dog 1	6	28.70	33.54	18.85	4.82	No anesthetic
Dog 2	3	17.86	20.83	16.63	4.82	No anesthetic
Dog 3	7.8	32.89	38.05	15.68	3.0	Barbital
Dog 4	17	56.43	65.14	15.43	3.5	Barbital
Dog 5	4.25	29.02	33.02	13.77	3.20	Barbital
Dog 6	5.1	27.09	29.99	10.70	5.45	Barbital
Dog 7	3.1	19.35	20.96	8.33	5.5	Barbital
Dog 8	2.5	15.86	17.09	7.71	7	Barbital
Cat 1	2.4	9.93	11.60	16.81	4	Barbital
Cat 2	3	14.31	16.44	14.88	3.5	Barbital
Cat 3	3	13.28	14.64	10.20	5.23	Barbital
Rabbit	2	7.095	8.19	15.45	4	No anesthetic
Guinea pig	490 gms.	3.22	3.74	16.00	4	No anesthetic

It will be noted that the unanesthetized animals showed the largest increase in cardiac area, although the oxygen per cent was not as low as in some of the other animals. This is probably due to the fact, particularly in the case of dogs, that they showed considerable excitement when the oxygen pressure became low; they would often bark and scratch on the glass doors of the respiration chamber. They were thus under the influence of exercise and in some ways their condition was analogous to the findings of Somervell of the Mount Everest Expedition. In the case of unanesthetized rabbits and guinea pigs, in spite of the fact that they were

fastened to the animal board, they nevertheless showed restlessness when the pressure in the respiratory chamber became rather low.

It will also be observed that dogs of different size were used; the smallest weighed 2.5 kilos and the largest 17 kilos. Incidentally two or three of the dogs were pups from 5 to 8 months old; they apparently withstood acute anoxemia as well as other animals.

As Takeuchi did his work on cats, it was thought well to try them as he reported rapid and pronounced cardiac dilatation in anoxemia. The results on the cats, however, did not materially differ from those in other animals.

By the methods described, in general, it may be said that the greater the degree of anoxemia, the greater the cardiac dilatation, up to a certain maximum as shown by the following examples:

	OXYGEN	CARDIAC AREA	INCREASE IN SIZE
Cat—2.8 kilos			
	per cent	*sq. cm.*	*per cent*
Normal		14.25	
Anoxemia	8.68	14.27	0
Anoxemia	6.89	15.09	5.89
Anoxemia	3.31	16.44	15.41
Dog—3.5 kilos			
Normal		22.09	
Anoxemia	8.27	22.54	1.95
Anoxemia	4.0	24.18	4.22
Anoxemia	2.5	27.86	20.66

The above results are somewhat contradictory to the findings of Takeuchi. He states that the principal effect on the size of the heart seems to be in the less extreme ranges of anoxemia. But Gremels and Starling working on the heart lung preparation found that, in general, the greater the degree of anoxemia the greater the cardiac dilatation. They interpret the results of Takeuchi as being due to vagal action. The question of the vagus in anoxemia will be dealt with in a later paper.

The present findings are not necessarily in conflict with those of Barcroft et al. The altitude at which the observations were made was 14,000 feet, corresponding approximately to 12 per cent oxygen; this altitude, however, caused some of the members to be rather severely afflicted with mountain sickness. No cardiac dilatation occurred at the above oxygen percentage by the methods employed in this work on anesthetized animals. The statement that in man anoxemia actually decreases the size of the heart is also of interest. I have some evidence in the case

of the dog that at reduced atmospheric pressure corresponding to about 9 to 11 per cent oxygen the cardiac area is diminished. The decrease is, however, very slight amounting to only 1 to 1½ per cent. While this lies within the experimental error, it was noticed in several animals and the reports on man seem to corroborate it. This phase of the problem, however, needs more careful quantitative work.

LeWald and Turrel working on man using less reduction in oxygen percentage than in my series, reported only slight and inconstant cardiac dilatation. In one or two cases the anoxemia was sufficient to induce fainting. They suggest that the failure to demonstrate cardiac dilatation might be due to vasodilatation in the splanchnic area, hence the roentgenogram was that of an empty heart. More work is needed on this particular point. It may also be mentioned again that the cardiac silhouette of man cannot be so accurately outlined as in the dog or cat, because in these animals the diaphragm lies lower and in many instances the entire heart shadow may be easily discerned. The errors due to the difference in the transverse diameter of the heart caused by the respiratory movements as described by these authors in man are less serious in the dog and the cat.

It has been reported by different writers that animals subjected to low atmospheric pressure in a chamber are greatly distressed by the gases in the stomach and intestine. The increased intra-abdominal pressure causes the diaphragm to encroach upon the space of the thoracic cavity and thus distorts the normal contour of the heart. This error was ruled out by the second described method of inducing anoxemia.

CONCLUSIONS

1. Dogs, cats, rabbits and guinea pigs, subjected to anoxemia (8.3 per cent to 2.5 per cent) showed acute cardiac dilatation as evidenced by the x-ray.
2. The greater the degree of anoxemia, the greater the cardiac dilatation up to a definite maximum.
3. There is a rather marked individual variation in this reponse of the heart to anoxemia, some animals showing a cardiac dilatation at a less reduction in oxygen percentage than others.

I wish to express my thanks to Dr. A. S. Loevenhart for the loan of his steel respiratory chamber and to Dr. R. S. Allen who assisted in a part of the technical work. I am also indebted to Dr. J. A. E. Eyster for his helpful suggestions and encouragement. I especially wish to thank Dr. Carlson for putting the equipment at my disposal, which made the work possible, and for his constructive criticism throughout the work.

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ELECTRICAL VARIATIONS AS AN INDEX OF PANCREATIC ACTIVITY[1]

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Evidence has been adduced that regulation of blood-sugar concentration by the pancreas is subject to vagus control, as shown by direct vagus stimulation (de Corral, 1918; McCormick, Macleod and O'Brien, 1923; Britton, 1925) and by use of pilocarpine (Clark, 1924, 1925). More recently La Barre (1927), by means of a temporary anastomosis between the pancreatic vein of a normal dog and the jugular vein of a depancreatized dog, has demonstrated a decrease in the blood-sugar of the depancreatized animal greater than can be accounted for by mere dilution. Using the same method of crossed circulation, Zunz and La Barre (1927) injected glucose into the blood stream of a normal donor and found a consequent reduction in the blood-sugar of an adrenalectomized recipient. By means of an "isolated head" preparation, they showed that an increase in the concentration of sugar in the blood supplying the head, produced an increase in the sugar reducing element in the blood of the pancreatic vein.

In the experimental work here presented we hope to show, by another method, that increase in the blood-sugar has a definite stimulative effect, either directly or indirectly, on the internal secretion of the pancreas. To this end we have examined the electrical changes induced in the pancreas by the injection of glucose.

The first application of the electrical method of investigation to the study of glandular phenomena was made by Bayliss and Bradford (1885) and later Bradford (1887), on the electrical changes in the submaxillary gland produced by nervous and chemical stimulation. Cannon and Cattell (1916) verified this work and investigated the electrical changes in the thyroid gland following stimulation of the cervical sympathetic nerves, and those produced in the pancreas after the injection of secretin. Gesell (1916, 1918, 1920), Bernard and Schulmann (1918), and Rabl (1922) have also studied the electrical changes of the submaxillary gland during activity. Anrep and Daly (1921) found a definite action current in the pancreas following secretin injection.

[1] The work was done under a Bullard Fellowship.

METHOD. Only healthy cats, weighing 2 kgm. or over, were used. The animals fasted for 18 to 24 hours before use and the stomach and small intestine were regularly found to be quite empty. It is essential that the islet tissue be in as near the resting state as possible before the observations are begun. The factors influencing the condition of the islet tissue are not definitely known, but in our work we found that the glycemic level should be as near normal as possible. Practically all the data presented here have been obtained from cats under amytal anesthesia. Page (1923) noted that amytal does not affect blood-sugar level, yet gives a satisfactory preparation. This has been confirmed by Britton (1925), Albritton (1924), and Edwards and Page (1924). A solution containing 65 mgm. of amytal per cc. was prepared as described by Page (1924) and 1 cc. per kgm. was injected intraperitoneally. With reasonable care the animal experienced no stage of excitement and the blood-sugar was found to be quite normal at the first observation. To minimize the amount of anesthetic required, the amytal was supplemented by local infiltration of 1 per cent novocaine in the region of operation. Chlorolose and decerebration were also tried but were found to be unsatisfactory.

The cat, anesthetized with amytal, was placed on an electrically heated pad and the mid-line of the abdomen infiltrated with novocaine from the xiphoid half-way to the pubis. The skin over the femoral vein and a small area in the groin were also infiltrated. The abdomen was opened by a mid-line incision in the anesthetized region, the omentum pushed upward, and a loop of duodenum with pancreas attached was brought into the wound. The common bile duct was identified and the duodenum opened longitudinally for about 2 cm. below the entrance of the bile duct. The papilla of Vater was identified by putting gentle pressure on the gall bladder. A glass canula was tied in the pancreatic duct and the intestine closed by continuous suture. The loop of duodenum was attached to a bent glass rod by means of ligatures, which fixed the pancreas beneath the incision, free from contact with other viscera. The right femoral vein was dissected out and kept moist by a normal saline sponge.

As a contact for the indifferent electrode, Waller (1903) and Cannon and Cattell (1916) have shown the advantages of using subcutaneous tissue. Cannon noticed an annoying tendency of the galvanometer to "drift" if the indifferent electrode is near the other one. The groin makes a very satisfactory place for the second electrode as there is no active tissue beneath it and the location is remote enough from the other electrode to check the "drift" tendency. Areolar and connective tissue were therefore exposed by a small incision in the left groin.

The electrical system consisted of a d'Arsonval galvanometer, a storage battery, a Wheatstone bridge, pole reverser, variable resistance, and the electrodes. The same electrode was always placed on the pancreas and

the galvanometer deflections recorded. In the first experiments the apparatus was used as illustrated (see fig. 1). Later, a duplicate complete set-up was added to record simultaneous currents from various organs. In this way the activity of the pancreas was compared with that of other organs, e.g., duodenum, muscle, submaxillary gland. Two types of electrodes were used during the course of these experiments. The first were made as described by Mines (1913) and consisted of a zinc electrode in saturated zinc sulphate solution. Such an electrode is subject to some disadvantages. It must be remade frequently; uniformity of resistance is difficult to obtain; and, most serious of all, the zinc sulphate tends to seep through to the tissues producing marked changes in the galvanometer deflection. The other electrode used was a modification of the original Ostwald (1900) calomel electrode, described by Alvarez (1924). Such

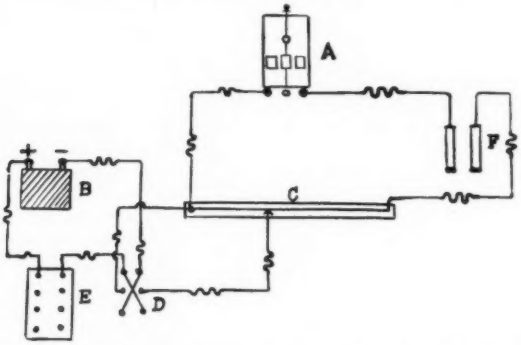

Fig. 1. *A:* D'Arsonval mirror galvanometer (undamped period 23 sec.; int. resist. 1490 ohms; amp. sensitivity 0.0163 m. amp. per cm. scale deflection). *B:* storage cell, 2 volt. *C:* slide wire contact of Wheatstone bridge. *D:* pole reverser. *E:* variable resistance. *F:* electrodes.

electrodes last indefinitely and have an average resistance of 1,500 ohms each. No difficulty with polarization currents has been encountered.

Blood was obtained from the right femoral vein by means of a syringe calibrated to deliver exactly 0.5 cc. Blood-sugar determinations were made by the method of Folin and Wu (1920), slightly modfied for 0.5 cc. instead of 2 cc. The loss of blood was reduced to a minimum.

Normal variation. At the beginning of an experiment the galvanometer often shows a tendency to "drift" in one direction or the other for several minutes before becoming relatively stationary. Thereafter this state of relative electric stability, with minor variations of 0.03 to 0.06 m. amp. on the galvanometer scale, may be maintained throughout the experiment if nothing is done to the animal. Usually the death of the animal is heralded by a shift of the galvanometer, sometimes as much as an hour

before death occurs. Figure 2 shows about an hour of normal variation, followed by a half hour in which pre-mortal changes are occurring. The respiratory rate is decreasing rapidly, the blood-sugar rises to a high level and the galvanometer changes rapidly. In all the experiments reported,

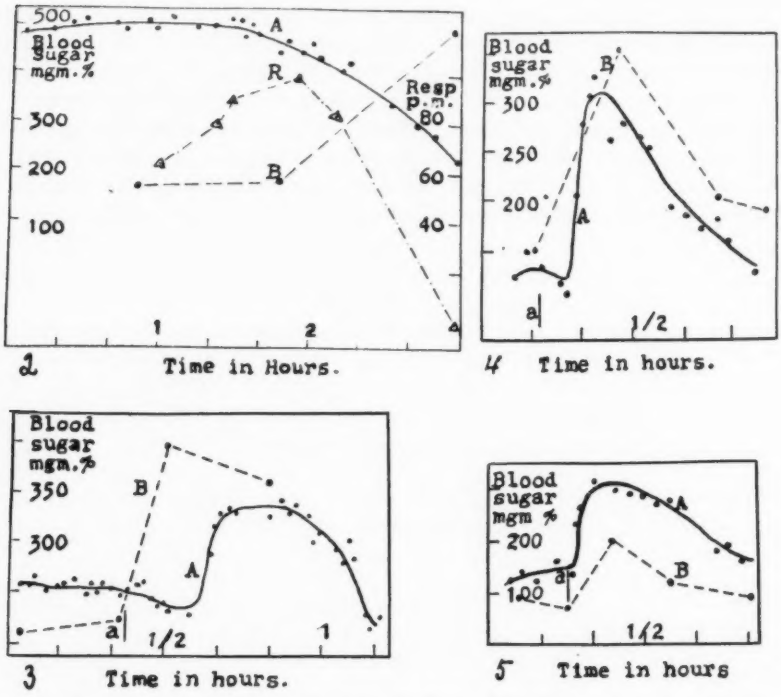

Fig. 2. Date: October 16, 1924. Cat, 1.6 kgm. Anesthetic: amytal, intraperitoneally. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *R:* respirations per minute.

Fig. 3. Date: May 22, 1924. Cat, 2.7 kgm. Anesthetic: amytal, 250 mgm. intraperitoneally. *A:* galvanometer deflection with electrode on the pancreas. *B:* blood sugar. *a:* 1 gram glucose i. v. (10 per cent solution; 0.37 gram per kgm.). Note: Galvanometer reading shown at two-minute intervals and average curve drawn through the points so plotted.

Fig. 4. Date: May 13, 1924. Cat, 2.5 kgm. Anesthetic: amytal, 205 mgm. intraperitoneally. *A:* deflection of galvanometer with electrode on pancreas. *B:* blood sugar. *a:* 1 gram glucose injected i. v. (10 per cent solution; 0.4 gram per kgm.)

Fig. 5. Date: October 30, 1924. Cat, 3 kgm. Anesthetic: amytal, 225 mgm., intraperitoneally. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *a:* 2 grams glucose, i. v. (20 per cent solution; 0.66 gram per kgm.)

TABLE 1

DATE	WEIGHT OF ANIMAL	GLUCOSE	LATENT PERIOD	PERIOD OF ACTIVITY	MAXIMUM DEFLECTION
	kgm.	*gm.*	*min.*	*min.*	*m. amp.*
3/25/24	2.1	0.5	1	30+	0.50
3/29/24	4.4	1.0	20	45+	0.37
4/ 8/24	2.6	0.25	1	60+	0.42
5/ 1/24	1.8	1.0	8	14	0.08
		1.0	2	12	−0.08
		1.0	2	24	0.08
		1.0	2	120+	0.23
5/ 3/24	2.2	1.0	18	30	0.13
5/13/24	2.5	1.0	6	34	0.26
5/17/24	2.5	1.0	1	30	0.36
5/20/24	1.7	1.0	2	78	0.37
5/22/24	2.7	1.0	16	30	0.59
7/19/24	2.1	1.0	12	180+	0.23
		1.0	10	30	−0.05
		1.0	10	20	0.05
		1.0	10	82	0.28
8/ 2/24	2.5	1.0	20	30+	0.33
		1.0	1	60+	0.49
9/23/24	2.3	1.0	1	23	0.26
		1.0	5	20	0.59
9/30/24	3.5	1.0	1	28	0.19
		1.0	1	10	0.10
10/ 7/24	3.0	1.0	4	60	0.39
10/14/24	2.7	2.0	18	32	0.13
		2.0	4	50+	0.62
10/30/24	3.0	2.0	1	67	0.62
11/ 4/24	3.3	2.0	1	60	0.18
11/ 6/24	3.1	3.0	1	30	0.59
11/13/24	2.6	2.0	4	36+	0.81
11/15/24	2.4	1.5	1	31	0.46
		1.5	2	6	0.06
		1.5	1	28	−0.16
		2.0	1	28	0.94
11/18/24	2.9	1.0	1	17	0.36
		1.0	1	10	0.33
11/25/24	1.2	2.0	20	20+	0.42
12/16/24	3.0	2.0	2	42+	0.62
1/29/25	2.6	1.0	1	12	0.26
		1.0	1	20	0.23
		1.0	1	10	0.26

a normal period of relative electrical stability was determined before any injections were made.

Effect of glucose injections. The injection of glucose was selected as the most probable method of exciting the internal secretion of the pancreas.

Figures 3, 4 and 5 show the usual effects on the electrical activity produced by the intravenous injection of glucose. The latent period was quite variable, but in 60 per cent of the cases (over 50 in number) it was 2 minutes or less, with only 17 per cent between 10 and 20 minutes. Possibly the variations were due to the condition of the animal, the action of the anes-

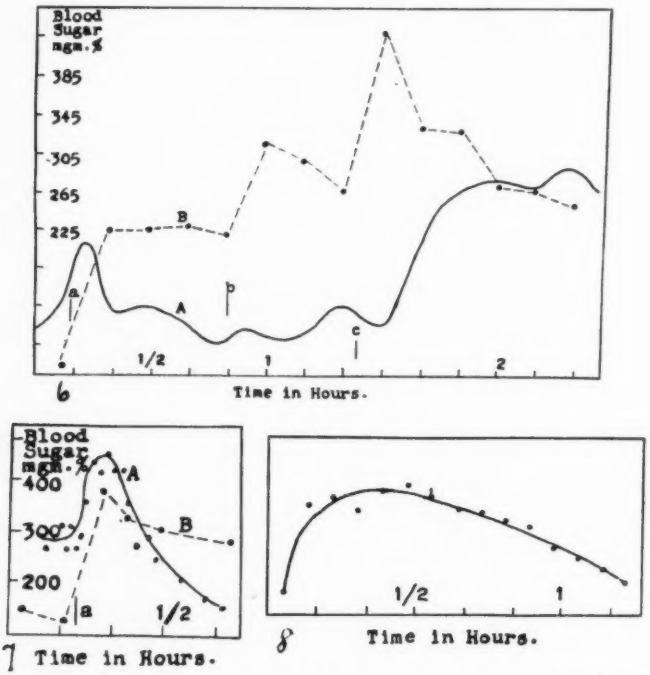

Fig. 6. Date: July 19, 1924. Cat, 2.1 kgm. Anesthetic: amytal, 205 mgm. intraperitoneally. *A:* deflection of galvanometer with electrode on pancreas. *B:* blood sugar. *a:* 1 gram glucose injected i. v. (10 per cent solution; 0.48 gram per kgm.) *b:* 1 gram glucose injected i. v. *c:* 1 gram glucose injected i. v.

Fig. 7. Date: October 30, 1924. Cat, 3 kgm. Anesthetic: amytal, 225 mgm. intraperitoneally. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *a:* 2 gram glucose, i. v. (20 per cent solution; 0.66 gram per kgm.)

Fig. 8. Average curve following glucose injection. Note: This curve represents the average current obtained following glucose injection in 40 consecutive experiments.

thetic, and the operative interference, all of which may be variable. The duration of the electrical change varied also, depending on the amount of sugar injected and the individual animal (see table 1). In about 20 per cent of the cases, the first or even second injection of glucose was not

followed by any marked electrical change. Figure 6 shows an experiment in which two injections of glucose caused little change in the galvanometer

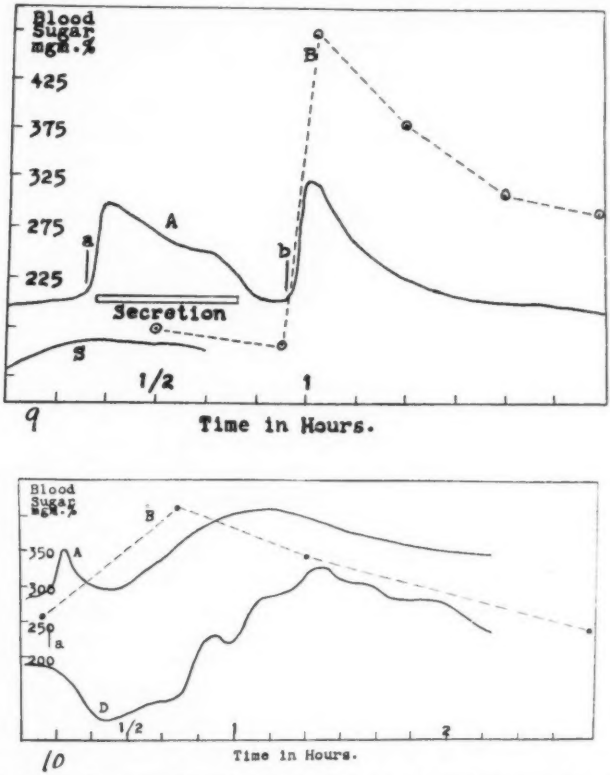

Fig. 9. Date: February 22, 1926. Cat, 2.6 kgm. Anesthetic: amytal, 240 mgm. intraperitoneally; novocaine infiltration of incisions. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *S:* galvanometer deflection with electrode on submaxillary gland. *a:* 5 cc. secretin solution i. v. *b:* 3 grams glucose i. v. (20 per cent solution; 1.19 gram per kgm.) Note: Pancreatic and submaxillary ducts cannulized and flow noted. Both vagi sectioned in cervical region at the beginning of experiment.

Fig. 10. Date: February 15, 1926. Cat, 1.8 kgm. Anesthetic: amytal, 100 mgm. intraperitoneally. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *D:* galvanometer deflection with electrode on duodenum. *a:* 3 grams glucose i. v. (20 per cent solution; 1.66 gram per kgm.) Note: Peristalsis and tonus waves observed in duodenum throughout the experiment.

reading. After the third injection, however, a rapid deflection occurred, accompanied by a more rapid fall in the blood-sugar.

Reversal of sign has been noted by a number of observers, among them Waller and Cannon. Figure 7 illustrates such an instance. It occurred but rarely during our observations.

Figure 8 represents a statistical study of 40 consecutive experiments in which glucose was injected. The curve was derived by averaging the galvanometer deflections obtained at five minute intervals after the injection of glucose. The form of the curve is materially altered from that found in the individual cases by the variation in the latent period in the different experiments.

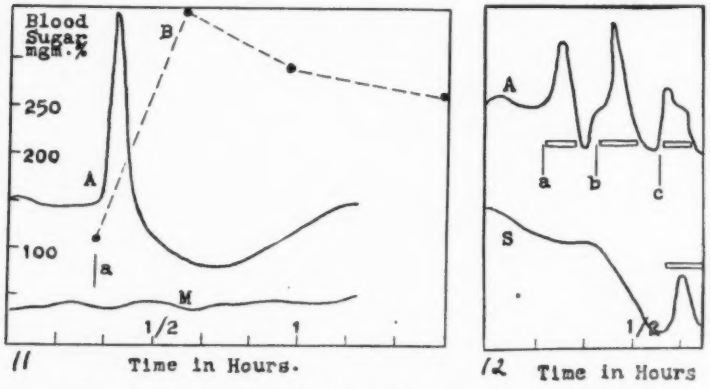

Fig. 11. Date: February 10, 1926. Cat, 2.9 kgm. Anesthetic: amytal intraperitoneally. Novocaine infiltration of incisions. *A:* galvanometer deflection with electrode on pancreas. *B:* blood sugar. *M:* galvanometer deflection with electrode on muscle. *a:* 3 grams glucose i. v. (20 per cent solution; 1.03 gram per kgm.)

Fig. 12. Date: February 19, 1926. Cat, 3.3 kgm. (pregnant). Anesthetic: amytal, 330 mgm. intraperitoneally. *A:* galvanometer deflection with electrode on pancreas. *S:* galvanometer deflection with electrode on submaxillary gland. *a:* 5 cc. secretin solution i. v. *b:* 5 cc. secretin i. v. *c:* 1 mgm. pilocarpine i. v. Note: Pancreatic and submaxillary ducts cannulized and flow noted.

In a few experiments, two other sugars were used, *i.e.*, fructose and galactose. Each of these produced curves quite similar to those obtained with glucose but with slightly longer periods of activity.

To decide whether the electrical change was due to stimulation of the islet tissue by glucose per se, or merely some physical effect of the injected material, a number of other substances were tried. Tap water, normal and hypertonic saline, urea and glycine have been repeatedly injected into the femoral vein with no associated electrical response in the pancreas.

The response is not due to a change in the rate of external secretion. In many experiments the pancreatic duct was cannulised and in no instance did external secretion follow injection of glucose. In confirmation of the

work of Cannon, and of Anrep and Daly, injection of secretin intravenously gave a typical action current coincident with a flow of external secretion from the pancreatic duct. Figure 9 depicts such an experiment. When the flow of pancreatic secretion ceased, glucose was injected. No further secretion (external) occurred, yet a curve somewhat similar in type was obtained. The most plausible explanation seems to us to be an altered activity of the islet tissue.

The deflection of the galvanometer is not due to the spread of electrical changes from the duodenum. The small intestine of cats under amytal anesthesia usually exhibits easily visible peristaltic waves. The double galvanometer set-up, described previously, revealed no correlation between the electrical activity of the pancreas and that of the duodenum. Figure 10 illustrates such an experiment.

That the response of the pancrease is specific is shown by figure 11. In this case the electrodes of the second galvanometer were placed on muscle. If the effect of the glucose injection was merely due to systemic disturbances, some parallelism between the effects on the two recording systems might be expected. There was none. In passing, it may be of interest to note the reversal of sign in this curve.

Other glandular tissue does not exhibit the same response as the pancreas. For these studies the submaxillary gland was chosen because of its accessibility and because its normal electrical response has been carefully observed. Figure 12 shows that secretin injections produce no definite changes in the submaxillary gland. Pilocarpine, however, excites the secretion of both pancreas and submaxillary gland and comparable curves are produced in each set-up after its injection.

We wish to acknowledge our indebtedness to Drs. W. B. Cannon, A. C. Redfield and R. P. Stetson for valuable assistance and suggestions rendered during the course of this work.

SUMMARY

A series of more than fifty experiments has shown that intravenous injection of glucose is usually followed, after a variable latent period, by a definite change in electrical state of the pancreas.

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